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TRE Environmental Strategies

March 6, 2019

Mr. Christopher Bittner
Standards Coordinator
Utah Dept. of Environmental Quality
195 N 1950 W
Salt Lake City, UT 84116

Dr. Gary Belovsky
Environ. Res. Center & Dept. Biol Sci.
University of Notre Dame
Notre Dame, IN 46556

RE: Results of Acute Arsenic Toxicity to Brine Fly Larvae

Mr. Bittner / Dr. Belovsky:

Enclosed is a copy of the final report entitled *Acute Toxicity of Arsenic to Juvenile* Ephydra cinerea *under Static-Renewal Test Conditions.*

We greatly appreciate this opportunity to provide our services to you. Please do not hesitate to contact us if you have any questions.

Sincerely,

Rami B. Naddy, Ph.D.
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David A. Pillard, Ph.D.
Manager / Senior Toxicologist
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Enclosure:

17001-474-029

University of Notre Dame
Notre Dame, IN

Acute Toxicity of Arsenic to Juvenile *Ephydra cinerea* Under Static-Renewal Test Conditions

Prepared by:

TRE Environmental Strategies

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A
Fort Collins, CO 80524

Document No. 17001-474-029

March 2019

Study Title

Acute Toxicity of Arsenic to Juvenile *Ephydra cinerea* in Laboratory Reconstituted Salt Water Under Static-Renewal Test Conditions

Study Period

Start: June 11, 2018 @ 19:00
End: June 15, 2018 @ 19:00

Performing Laboratory

TRE Environmental Strategies, LLC
100 Racquette Drive, Unit A
Fort Collins, CO 80524

Telephone: (970) 416-0916
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Laboratory Project ID

17001-474-029

SUMMARY

Study Directors	Rami B. Naddy, Ph.D. David A. Pillard, Ph.D.
Test Facility	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 (970) 416-0916
Location of Data	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 (or an offsite storage location)
Test Substance	Sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$; Alfa Aesar Lot #T02A076; TRE #C17-021)
Subject	Static-Renewal Toxicity Test
Test Dates	Initiated: June 11, 2018 @ 1900 Terminated: June 15, 2018 @ 1900
Length of Study	96 Hours
Test Species	*Ephydra cinerea*
Source of Organisms	Notre Dame University
Age of Test Organisms	Larva, 3rd Instar (~ 30 d)
Test Concentrations	Nominal Arsenic Concentrations: 0; 12,800; 32,000; 80,000; 200,000; 500,000 µg As/L
Dilution Water	Laboratory Saltwater Reconstituted Water (rGSL; RW #13316 & 13340) Target: Salinity ~ 120 ppt
Results	96-Hour LC_{50} Based on Measured Arsenic Concentrations: 116,600 µg total recoverable As/L; 95% C.I. (50,110 – 138,300) 113,700 µg dissolved As/L; 95% C.I. (46,800 – 134,400)

Sponsor and Laboratory Information

Sponsor	Environmental Research Center University of Notre Dame 97 Galvin Life Sciences Center Notre Dame, IN 46556
Project Officer	Gary E. Belovsky, Ph.D. (574) 631-0172
Testing Facility	TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A Fort Collins, Colorado 80524 Fax: (970) 490-2963
Study Directors	Rami B. Naddy, Ph.D. (970) 416-0916 email: naddyrb.tre@gmail.com
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Introduction

This report presents the results of a study conducted to determine the acute toxicity of arsenic (as sodium aresenate heptahydrate) to larval *Ephydra cinerea* in a laboratory reconstituted salt water under static-renewal test conditions. The ultimate goal is to use these data to aid in the development of ambient water quality criteria for metals for the protection of species in the Great Salt Lake.

Methods followed the Work Plan for Great Salt Lake Toxicity Test, Version 8, October 23, 2016; Dr. Belovsky, University of Notre Dame, except where noted. The target water used in these studies was prepared to mimic Gilbert Bay water (see work plan; Appendix A), with a salinity of 120 ppt.

All toxicity tests were conducted at TRE (Fort Collins, CO). Chemical confirmation of arsenic was carried out at ALS Environmental (Kelso, WA; primary analytical laboratory).

METHODS
Test Media

The artificial reconstituted Great Salt Lake (rGSL) water was used in holding and testing conditions for the brine fly larvae. It was prepared as follows with ASTM Type I (Milli-Q®) water (ASTM 2012):

- Crystal Sea marine mix: 50.95 g/L
- Potassium chloride (KCl): 2.99 g/L
- Magnesium sulfate ($MgSO_4$): 6.19 g/L
- Sodium chloride (NaCl): 65.77 g/L

Salts were added to Milli-Q® water in a 20 L carboy and stirred to mix salts. Analytical results for rGSL water prepared in the same manner as the batch used in this study are reported in Appendix B. The laboratory reconstituted salt water had an initial salinity of ~120 ppt.

Test Organisms

Test organisms were *Ephydra cinerea* larva obtained from cultures at the University of Notre Dame. The larvae were obtained as late 2nd and 3rd instars on June 6, 2018. The larvae were shipped in high-density polyethylene (HDPE) containers containing a dense solution of the larvae's food, the green alga *Dunaliella viridis.* The larvae and algae solution were transferred to 2-L, polypropylene holding chambers (contents of each HDPE bottle were placed in a separate holding chamber). Approximately 1 L of rGSL water was added to each holding chamber, the chambers were covered, and the water was gently aerated. Small plastic screens (~1 mm opening) were added to each holding chamber to provide a resting substrate for the larvae. Larvae were kept in the pre-test holding conditions for a minimum of four days before test initiation.

The food source for the *E. cinerea* was the salt water alga *D. viridis*, which was cultured at ~10°C with 16:8 h light: dark photoperiod under constant aeration. The media used to culture *D. viridis* consisted of the following added to Milli-Q® water:

- Morton's Water Softener Salt (80 ml/L)
- Crystal Sea marine mix (53.3 ml/L)

The media was mixed well and filtered (Buchner funnel) through a 110-mm Whatman® #4 filter paper and sterilized using a pressure cooker. Nutrients (1-3 ml) were added to the *D. viridis* cultures 1-2X per week. The nutrient solution consisted of Milli-Q® water (80 ml), Blue solution (10 ml), and P/N solution (10 ml). The Blue solution consisted of 41.7 g/L Hydrosol, 27.5 g/L calcium nitrate, 22.1 g/L ammonium nitrate and Milli-Q® water. The P/N (phosphorus/nitrogen)

solution consists of 8.79 g/L monopotassium phosphate, 20.0 g/L ammonium nitrate, and Milli-Q® water.

Pre-Test Conditions

In the pre-test holding conditions, received larvae were placed in 2-L polypropylene chambers. The density of *D. viridis* in each chamber was at least 40 µg chlorophyll/L. Organisms were held at 20°C with a 16 h dark:8 h light photoperiod. The holding chambers were aerated with gentle bubbling. The initial water (shipping water plus ~ 1 L of rGSL water at 120 ppt) was partially replaced on day 2 of the holding period. Additional *D. viridis* from TRE cultures was added both in suspended form as well as on filters (Whatman® GF/F) which were weighted down with flat, glass marbles.

Test Conditions

The chemical used in testing was sodium arsenate heptahydrate (Na_2HAsO_4-$7H_2O$; Alfa Aesar, Lot #T02A076, TRE # C17-021). Individual test solutions were prepared by addition of the appropriate volume of the arsenic stock solution (8,000 mg/L as As) to rGSL water. Once each test concentration had been prepared, it was inverted at least three times to ensure even distribution of the metal. Approximately 150 ml of solution were poured into each test chamber (n = 5). Test chambers were 12 oz Pro-Kal® polypropylene dishes[1].

After test solutions of the correct concentration were poured in the appropriate test chambers, food was added to each for equilibration. Algae (*D. viridis*, ~500 µg Chl *a*/L) was collected on a Whatman® GF/F microfiber filter using vacuum filtration. The concentration of algae became more dense towards the bottom of the culture flask, but approximately the same amount of algae was collected on each filter based on volume filtered and filter saturation (i.e., no more solution could be drawn through the filtration apparatus and therefore the filter was saturated with algal cells). A filter containing the collected algae was placed into each test chamber containing the test solutions, and weighed down with a flat, glass marble. The solutions were allowed to equilibrate for ~3 hours prior to use in testing.

After the equilibration period, 10, 3rd instar larvae were placed in each chamber along with a resin fiber pad (3M™ Scotch-Brite #86) that had been split lengthwise to yield a thinner pad. This provided a substrate to which the larvae could cling (using proleg claws), but was thin enough so larvae could be seen and mobility/morbidity could be more easily determined. The resin pad and filter were weighted with a micro-binder clip (19 mm wide). Test chambers were placed in a randomized design in a temperature-controlled water bath (20°C). The photoperiod was 16 h light:8 h dark using fluorescent lighting. The test chambers were covered during testing to minimize evaporative loss and prevent foreign material from entering the test chambers.

[1] Polypropylene test chambers were used instead of HDPE test chambers as mentioned in the work plan

The study consisted of a 96-h exposure period in which *E. cinerea* were exposed to different concentrations of arsenic. The test solutions were renewed after 48 h[2] with test solutions that had been prepared and equilibrated with *D. viridis* in the same manner as at test initiation. Surviving organisms were verified at 48 h (test solution renewal) and 96 h (organisms were not handled on days 1 and 3).

Further detail is provided in Table 1 below.

Table 1. Additional Test Conditions in the Toxicity Test

Type	96-h Static-Renewal Acute (renewal at 48-h; see deviation to work plan)
Test Endpoints	Mortality (no response to stimulus); see deviation to work plan
Test Concentrations (nominal)	0 (control), 12800, 32000, 80000, 100000, and 500000 μg As/L
Quality Criterion	≥90% control survival
Analytical Confirmation	Test initiation (new): Dissolved and total recoverable samples for each treatment Test renewal (new and old): Dissolved and total recoverable samples for each treatment Test termination (old): Dissolved and total recoverable samples for each treatment
Arsenic Analyses	ICPMS (EPA Method 200.7) ALS Environmental – Primary Analytical Laboratory
Statistical Analyses	96-h median lethal concentrations were determined using CETIS 2014

[2] The work plan mentioned renewals every 24-h although preliminary analytical work indicated that 48-h renewals were sufficient for analytical and biological needs.

RESULTS

The initial characteristics of the rGSL water for a representative batch are reported in Table 2.

Table 2. Initial Dilution/Control Water Characterization

Batch No.	pH	Hard. (mg/L)[a]	Alk. (mg/L)[a]	Spec. Cond. (µS/cm)	Salinity (ppt)
13316	7.8	11,800[c]	111[c]	139,100	122

[a] As $CaCO_3$
[b] Total residual chlorine
[c] Measured in rGSL Batch 13090 (12/07/17)

The batch of rGSL water was not analyzed for dissolved and total recoverable metals as in previous batches due to the consistency in results in earlier studies. Refer to the two previous studies that reported the dissolved and total recoverable metals for the rGSL water (see TRE report #s: 14001-474-012 and 14001-472-018). A summary of the analyses can also be found in Appendix B.

The range of water quality parameters measured during the toxicity test is provided in the table below (Table 3). Overall, the pH, dissolved oxygen, conductivity, and temperature were similar among treatments.

Table 3. Physical and Chemical Data Measured during the Toxicity Test

Treatment (Nominal Test Conc., µg As/L)	pH		Dissolved Oxygen (mg/L)		Conductivity (mS/cm)		Temperature (°C)	
	Low	High	Low	High	Low	High	Low	High
0 (Control)	7.8	8.9	4.3	4.8	129.1	139.1	22	22
12,800	7.7	8.9	4.2	4.8	127.8	139.8	22	22
32,000	7.7	8.8	4.3	4.8	128.1	135.2	21	22
80,000	7.7	8.8	4.3	4.8	127.9	134.5	22	22
200,000	7.7	8.6	4.3	4.7	127.5	141.2	21	22
500,000	7.7	8.4	4.4	4.8	128.3	132.9	21	23

Analytical Confirmation

Samples were collected for total and dissolved arsenic analyses from new and old test solutions as outlined in the Methods Section. Average concentrations for all treatments are presented in Table 4.

Table 4. Measured Arsenic Concentrations

Nominal Conc. (µg As/L)	Avg Total Rec. (µg As /L)	% of Nominal	Avg. Dissolved (µg As/L)	% of Nominal	Diss. / Tot. (%)[a]
0 (Control)	53 U	--	53 U	--	
12,800	9,790	76	9,510	74	96
32,000	24,725	77	24,250	76	99
80,000	61,200	76	59,925	75	98
200,000	151,250	76	146,500	73	96
500,000	347,000	69	322,750	65	100

[a] Initial samples only (Appendix B)

Note: Refer to Appendix B for a summary of analytical results

U = less than the MRL / MDL (which is reported)

Organism Response

The definitive test was initiated June 11, 2018 at 1900 hours and was terminated on June 15, 2018 at 1900 hours. *Ephydra cinerea* survival at 48 h and 96 h is presented below (Table 5).

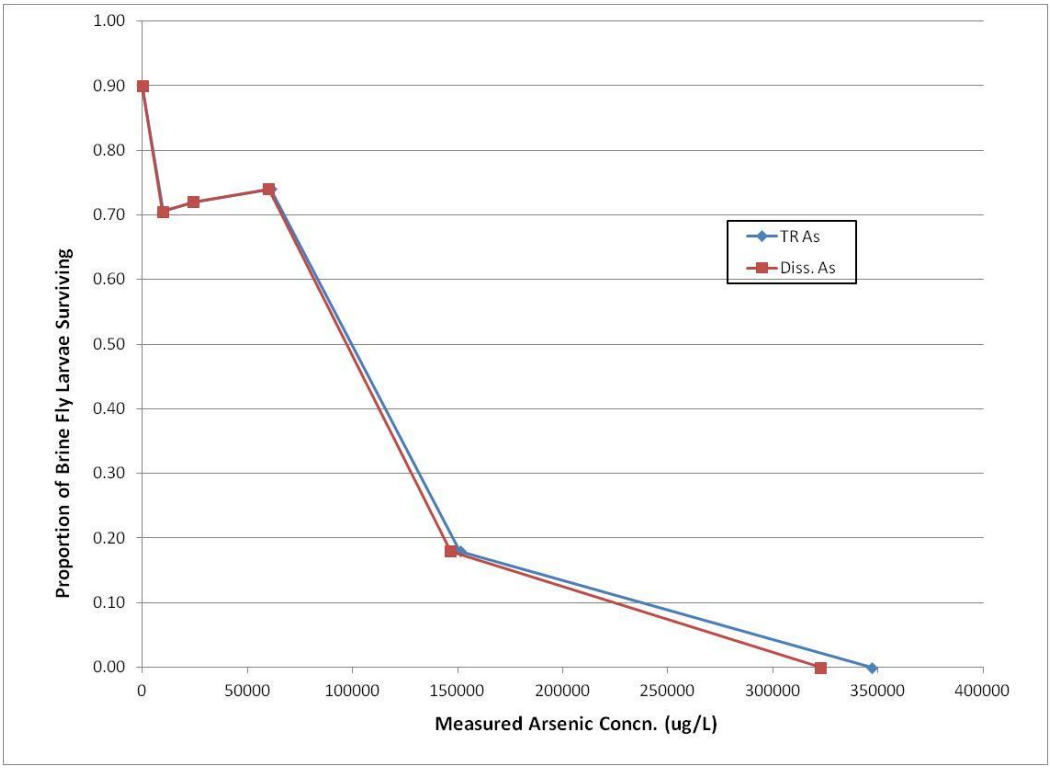
Table 5. Survival (%) of *Ephydra cinerea*

Nominal Conc. (µg As/L)	% Survival		
	0 h	48 h	96 h
0 (Control)	100	98.0	90.0
12,800	100	84.4	70.5
32,000	100	88.0	72.0
80,000	100	94.0	74.0
200,000	100	44.0	18.0
500,000	100	8.0	0.0

Note: See Appendix C for a copy of raw data

Survival versus measured arsenic concentration is shown in Figure 1.

Figure 1. Proportion of *Ephydra cinerea* Surviving at 96 hours vs Measured Arsenic



The calculated 96 hour LC_{50} values for *E. cinerea* are provided below for total recoverable and dissolved arsenic (Table 6).

Table 6. 96 hour Median Lethal Arsenic Concentrations (µg/L)

Endpoint	Total Rec. (µg As/L)	Dissolved (µg As/L)
LC_{50}	116,600	113,700
95% C.I.	50,110 – 138,300	46,800 – 134,400
Method	Probit	Probit

References

ASTM. 2012. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E 729-96. Annual Book of ASTM Standards, Volume 11.05, Section 11, Water and Environmental Technology.

Belovsky, G. Work Plan for Great Salt Lake toxicity Tests, Version 8. October 23, 2016. Environmental Research Center and Department of Biological Sciences. University of Notre Dame.

CETIS. 2014. Comprehensive Environmental Toxicity Information System. User Guide (version 1.8.7). Tidepool Scientific, LLC. McKinleyville, CA.

STATEMENT OF PROCEDURAL COMPLIANCE

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.

David A. Pillard, Ph.D.
Study Director

03 March 2019
Date

STATEMENT OF QUALITY ASSURANCE

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data generated at TRE.

Quality Assurance Unit

March 5, 2019
Date

APPENDIX A

WORK PLAN

Work Plan For Great Salt Lake Toxicity Tests, Version 8
October 23, 2016

Gary Belovsky
Environmental Research Center & Department of Biological Sciences
University of Notre Dame

Introduction:

Great Salt Lake (GSL) is a unique ecosystem, the fourth largest (largest in the western hemisphere) hypersaline lake in the world[16]. Invertebrate life in the GSL is relatively species poor due to the high salinity of the lake and is dominated by brine shrimp (*Artemia franciscana*) and brine fly larvae. Two or more species of brine flies occur in the GSL with *E. cinerea* the most abundant by several orders of magnitude. Thus, *Artemia franciscana* and *Ephydra cinerea* are the dominant grazers in the GSL food web in Gilbert Bay (South Arm) of Great Salt Lake, and are the focus of this study. These invertebrates are very abundant and are the main source of food for many resident and migrating water birds, which have important ecological and conservation value. Some of these water bird species are threatened or endangered or have other legal protections.

The purpose of this project is to determine toxic levels of copper, arsenic and lead, to brine shrimp and brine fly larvae so that water quality criteria (WQC) can be developed for GSL as described in the State of Utah Division of Water Quality (UDWQ) Great Salt Lake Strategy[22]. These potential pollutants were identified as the highest priorities by UDWQ after public feedback and comments.

We will conduct acute toxicity tests of the above substances for brine shrimp and brine fly larvae (*E. cinerea*) and develop a plan of work to assess chronic toxicity of these trace elements. *E. cinerea* has been chosen as a test species because of its local abundance end ecological dominance and it has been successfully cultured in the laboratory. American Society for Testing and Materials (ASTM) has not sanctioned standard methods for toxicity testing with either of these species. However, other ASTM-approved methods for invertebrates are established and will be used as a guide for the conduct of the toxicity testing proposed here.

In this work plan we present the protocols that lead to uniform rearing of the brine shrimp and brine fly larvae, as well as production of control and test (pollutant) solutions. We then focus on range-finding and acute testing, which must be completed before proceeding with the ecologically more relevant chronic testing (survival and reproduction with life-time exposure to the pollutant), which will be detailed in a future work plan. Range-finding establishes the range of concentrations for each pollutant that produces short term (96 hour) toxicity (assessed by mortality and growth). Once the range for each pollutant has been established, acute tests will be conducted to establish the concentration of each pollutant that will produce 50% mortality over 96 hours of exposure (LC50). For this project, the Belovsky laboratory has primary responsibility for developing the culturing methods for the brine flies and brine shrimp and will provide brine flies to the bioassay laboratory. The bioassay laboratory has primary responsibility for conducting the toxicity tests for both species –*Artemia franciscana* cultured at the bioassay laboratory per protocols provided by the Belovsky lab, and *Ephydra cinerea* which will be provided by the Belovsky lab.

Source animals and rearing conditions:

Given the uniqueness of Gilbert Bay, Great Salt Lake compared to other aquatic environments for which ecotoxicology studies have been developed, it is critical that our toxicology studies provide results that are applicable to the lake's environment. The environmental conditions found in the Great Salt Lake in April – October (the time when brine shrimp are present) over a 20 year period (1994 – 2013[16, 17]) are summarized in the table below.

Consistency of *Artemia* will be ensured by using a single batch of brine shrimp cysts (resting eggs) that have been commercially harvested from GSL. Brine fly larvae will be obtained from a colony maintained at UND, where GSL was the source of individuals starting the colony and the colony has been in existence for approximately two years (6 – 8 generations). The 3rd instar were selected because this is the longest and final larval stage when the most growth and development occurs[20]. This stage can be easily collected without damaging them from the rugose surface of bioherms on which they are raised in the laboratory colonies. Furthermore, this life stage is long enough to support shipping the larvae and robust enough to experience <10% mortality in transit.

Both species will be reared in environmental chambers that maintain temperature ($\pm$ 1°C) and a light:dark cycle (16:8, ~summer day using full spectrum lighting) using the following protocols:

> **Brine shrimp** will be hatched in 10 gallon aquaria at a salinity of 45 ppt, the optimum for hatching and hatchling survival. Nauplii will be used in the bioassays as it is thought that they are most susceptible due to their small size and less developed exoskeleton. Twenty-four hours after hatching, the nauplii will be transferred to artificial GSL water (see below). Over the initial 2 days post hatch, individuals are fed ad libidum a high quality phytoplankton (*Dunaliella* sp.: 40 µg Chl_a/L/2 days) maintained in culture.
>
> **Brine fly larvae** will be reared in plastic containers (60 cm X 60 cm X 25 cm) that contain 12 cm of water that is maintained at average lake conditions specified above at the University of Notre Dame. Gravel and GSL bioherm (approximately 30 cm X 15 cm X 15 cm) serve as a substrate for larvae and pupae, and an above water platform is provided to emerging adults for resting and mating. Larvae will be fed ad libidum (pupae and adults do not feed) a high quality food (*Dunaliella* sp.: 40 µg chl_a/L/2 days) from a colony established from the GSL. Notre Dame personnel ship late 2nd or 3rd instar larvae based on size (FedEx overnight) with a resin fiber pad (3M ™ Scotch Brite #86) for attachment and food (*Dunaliella* sp.) in a plastic bag with head space and bags in a cooler with ice. Larvae will then be acclimated in artificial GSL water (see below) for a minimum of four days prior to the beginning of each test.

Dilution Water:

Artificial reconstituted GSL water (rGSL) (Table 1, salinity = 120 ppt mass/volume) will be made to duplicate Gilbert Bay water as close as practical. This specific artificial reconstituted water was selected based on considerations of the data quality objectives (see Appendix 1).

The rGSL attempted to match the average concentrations of salts that are shown in Table 2 based on Utah Geological Survey measurements from Gilbert Bay[18] (Table 2). Initially, no attempt will be made to mimic the dissolved organic content (DOC) of Gilbert Bay. DOC in Gilbert Bay water has been reported to reduce the toxicity of copper and other metals compared to artificial Great Salt Lake water[3] (Brix et al 2006). Using Gilbert Bay water instead of artificial water would duplicate the DOC concentrations at the time the water was collected but the representativeness over time is unknown. For instance, reported DOC concentrations include 7 mg/L to 42 mg/L[3,2]. Amending the rGSL with DOC was considered but was primarily rejected by UDWQ because of the lack of data to support determining an appropriate target DOC concentration.

For the acute tests, rGSL will be made using reagent grade Crystal Sea™ Bioassay Laboratory Formula Marinemix® (Marine Enterprises, Baltimore, Maryland). Marinemix is approved by EPA for toxicity testing[19]and American Chemical Society (ACS) grade salts to deionized water in 20L Nalgene® carboys. Facility Deionized filtered water will be used. pH will be maintained at 7.9 ± 0.2 via the addition of 0.1N nitric acid or 0.1N sodium hydroxide as necessary. Filtered triplicate samples will be collected from each batch to verify that the salt concentrations and pH are within acceptable ranges.

Recipe	g/L
MarineMix	50.95
KCl	2.99
$MgSO_4$	6.19
NaCl	65.77

Table 1. Reconstituted Great Salt Lake water used for acute toxicity testing.

Constituent	Great Salt Lake Average	rGSL	% match
Na	42.0	41.53	98.54
Mg	4.4	3.19	100.77
Cl	74.0	69.33	100.48
K	2.8	2.14	100.40
Ca	0.3	0.62	99.73
SO_4	9.3	8.85	99.95
HCO_3	0.4	0.22	100.14
CO3	No data	0.02	No data

Table 2. Reconstituted Great Salt Lake (rGSL) water nominal concentrations compared to Utah Geological Survey average Great Salt Lake (Gilbert Bay) Concentrations from 1966-2013[18] .

Prior to initiating testing using organisms, the rGSL will be characterized for trace metals and metalloids and the analytical results from the copper range finding test (Appendix 2) will be confirmed. To characterize the rGSL, the concentrations of antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc will be measured. To confirm the analytical results from the copper range finding test, a sample of the

rGSL will be spiked with 450 ± 50 µg/l of copper as $CuCl_2$. The salinity of the rGSL may need adjustment to achieve the target salinity of 120 ppt after spiking with $CuCl_2$. The copper-spiked rGSL will be stirred to mix and both filtered (0.45µm) and unfiltered samples collected and analyzed. The measured concentrations in the filtered samples should be at least 70% of nominal. If measured concentrations are less than 70% of nominal, additional experiments will be pursued to determine the fate of the copper spikes.

Test solutions (treatments):

The pollutants chosen for these studies were selected by UDWQ after soliciting public comment. American Chemical Society (ACS) reagent grade chemicals will be employed. To maximize comparability of test results with published ecotoxicology studies[2-14] that have been conducted with other aquatic invertebrates, the following chemicals will be used to produce test solutions:

- As (arsenic) as sodium arsenate;
- Cu (copper) as copper chloride;
- Pb (lead) as lead nitrate.

Stock solutions of each trace metal will be made fresh for each of three rounds of acute toxicity testing as recommended by US EPA. Solutions will be made in Teflon sample bottles and solution concentrations will be verified at the beginning and end of each exposure 24-hour day of the bioassay (see details below).

Range finding tests:

For each pollutant to be tested, a preliminary range finding test will be conducted to establish concentrations to be used in the acute tests so that well resolved concentration-response (mortality or immobility) curves result. Initial range finding will be conducted with 10-fold increases in dissolved concentrations (e.g., 0 (controls), 10, 100, 1,000, 10,000, 100,000 µg of the pollutant/L) to ensure that we can develop well-resolved concentration response curves. Exposure to each concentration will be tested in triplicate for each test species over a 96 hour period on a static renewal basis (100% water changes occurring at 24, 48 and 72 hours), as follows:

Brine shrimp – 48 hour old nauplii will be used for all acute tests (including preliminary range finding tests). Test chambers will be 250 mL HDPE beakers containing 150 mL of test solution. Nauplii will be hatched in Marine Mix formula at 45ppt and then transferred to MarineMix at 120 ppt for tests. The shrimp nauplii will be fed *Dunalliela* from culture at a rate of 190 µg Chl_a/L/day. These feeding rations were determined to be the minimum rations required to achieve ≥ 90% control survivorship in feeding trials were conducted at UND. The feeding trials were conducted using 5 replicate treatments using the same rGSL, temperature, number of nauplii and container size as the acute tests. The initial survival was <90% and the food was increased by 50% until >90% survival was observed. Food was decreased from this rate to the midpoint of the next lowest feeding rate and the trials repeated until the minimum amount of food resulting in ≥ 90% survival was determined (190 µg Chl_a/L/day).

For the acute tests, feeding rates will be measured by taking at least three (3) Chl_a measurements of the feeding solution with the fluorometer (equivalent FSUs, fluorescent signal units, for the

necessary µg Chl_a feeding level) will be made after mixing vigorously between measurements. The test conditions are summarized in Table 3.

Brine fly larvae – 3rd instar larvae will used in all acute and range finding tests. Ten individuals per replicate will be held in a 250 ml HDPE beaker containing 150 ml of test solution. The larvae will be fed *Dunalliela* from culture at the minimum necessary to achieve rate ≥ 90% control survivorship as determined at UND using the same feeding trial methods as for nauplii. This food level is attained by vacuum filtering at ~1 atmosphere 150 mL of 490 µg Chl_a/L through a glass microfibre filter (Whatman Catalog # 1825 024) 50 mL at a time. The FSU of the filtrate will be measured and recorded to verify that the phytoplankton were captured on the filter. The filter paper will be placed phytoplankton-side up in the bottom of the empty beaker. A piece of a resin fiber pad (3M ™ Scotch Brite #86) weighted with a paperclip will cover the top of the filter and provide a substrate for the larvae. The larvae are then added to the beaker.

For brine shrimp and brine fly larvae, beakers will be acid washed (5% HNO_3), rinsed several times with deionized water and dried under a laminar flow hood prior to use. Each beaker will be covered with parafilm® to reduce evaporative water loss and associated changes in salinity and pollutant concentration. The resulting headspace in each beaker will provide ample gas exchange (O_2 and CO_2) on a 24 hour basis for the animals between daily treatment solution changes. Individuals will be randomly assigned to treatment groups, and the placement of beakers in the incubators will be randomized as well. pH, DO, and conductivity in each beaker will be checked daily, as well as in a "monitoring replicate" (no test organisms present) for each concentration.

Dead and immobile individuals will be recorded daily. Dead individuals are those that are immobile and unresponsive to stimuli (touched with pipette), while immobile individuals are not observed to move until touched with the pipette. Dead individuals are removed by pipette daily at the time of treatment solution change. After the sampling and methods for measuring concentrations in the test solutions are verified to be accurate, analytical chemistry of the treatment solutions will not be conducted in the range testing work, because it is only necessary to establish which treatment solutions produce mortality or immobility and to reduce analytical costs.

Acute Toxicity Assays:

Concentration ranges for acute assay treatment solutions – Based on range finding tests, we will run appropriate dilution series such that we can make robust statistical estimates of LC50 concentrations for each species. Six test concentrations (including controls) will be used for each species. If it is determined that the concentrations required to elicit mortality exceed the solubility limit of the metal under our test conditions, acute testing will be halted and the pollutant will proceed to chronic testing.

Acute assay protocols – Acute assay protocols are summarized in Table 5. Assays will be executed as described in range finding tests (see above) with the exception that 5 replicates will be used per test concentration (as opposed to 3 for the range finding tests). Fresh test solutions will be made daily and verified (see below). Tests will be run on a static renewal basis

with a 100% water change and the removal of dead test organisms occurring on a daily basis. At the termination of each test, surviving organisms will be counted, and dried on pre-weighed filter paper for analysis of growth differences between treatments. Mortality data will be analyzed via probit analysis.

Analytical chemistry. Test exposure concentrations will be verified daily at the beginning and end of each 24 hour exposure period to ensure that we are accurately characterizing exposure conditions. When possible and supported by the range finding results, brine flies and brine shrimp will be tested simultaneously to reduce analytical costs. Table 4 illustrates the number of analytical samples (88) needed assuming that both brine shrimp and brine flies are tested simultaneously with 5 exposure concentrations plus the control. Three of these exposure concentrations are assumed to be identical for both brine flies and brine shrimp and 2 of the exposure concentrations are unique to both brine flies and brine shrimp.

Note that the addition of *Dunaliella* as a food source for *Artemia* nauplii and brine fly larvae will require that post exposure water samples are filtered to remove particulates (*Dunaliella).* Because the addition of live cells (*Dunaliella*) as a food source is likely to decrease the dissolved concentrations of the test chemicals, all statistical analyses will be based on the geometric mean of the initial (pre-exposure) and final (post-exposure) dissolved concentrations. Treatment solution samples will be filtered through acid washed (5% HNO_3) 0.45 µm syringe filters that have had 3 volumes of sample water passed through them prior to retaining the sample in the appropriate acid washed (5% HNO_3) sample tubes (500 ml). Comparisons of pre-exposure test solutions will be made between filtered and unfiltered samples to describe the relationship between total and dissolved metals under our test conditions. Samples will be preserved and kept at 4^oC in the dark in 15mL conical tubes for As, Cu and Pb samples which will be stabilized with Omnitrace nitric acid or as instructed by the analytical laboratory

Acute assay data analyses – Two toxic endpoints will be recorded - mortality and immobility. Records of daily deaths and immobility will be recorded, but analyses will be conducted on overall mortality and immobility over the entire 96 hour assay period for a given pollutant concentration. Measures of mortality and immobility for a given pollutant concentration will be presented relative to the respective values observed in the simultaneous controls (no pollutant). For an assay to be considered successful, $\geq$ 90% of individuals in the control must survive.

With the above measures, the concentration-response (mortality or immobility) curves will be developed for a given pollutant and organism. These curves will be calculated via standard analytical procedures with diagnostic checks for homogeneity of variances using standard statistical packages . With the concentration-response curves, a number of toxicity effects for a pollutant can be estimated:

1) LC50 and EC50 is computed as the concentration eliciting 50% mortality (LC50) and 50% immobility (EC50) relative to the organism's control values.

2) Lowest concentration (LOEC) affecting mortality and immobility is defined as the first test concentration to produce a statistically significant increase in mortality or immobility relative to control values.

3) No effect concentrations (NOEC) is the next lowest concentration tested relative to the LOEC.

Data archiving -- all water chemistry, QA/QC data, and toxicity (mortality and immobility) data will be provided to UDWQ and made available to any interested parties

	Brine shrimp	**Brine Flies**
Test Type	Static renewal	Static renewal
Test Duration	96-hr	96-hr
Temperature	20 +/- 1 °C	20 +/- 1 °C
pH	7.9 +/-0.1; adjusted with 0.1N nitric acid and/or 0.1N sodium hydroxide as needed	7.9 +/-0.1; adjusted with 0.1N nitric acid and/or 0.1N sodium hydroxide as needed
Salinity	120 ppt	120 ppt
Photoperiod	16 hr light/8 hr dark	16 hr light/8 hr dark
Test Concentration or Dilution Series	5 exposures + control (40% dilution series TBD by range finder test)	5 exposures + control (40% dilution series TBD by range finder test)
Test Chamber Size	250 ml	250 ml
Test Solution Volume	150 ml	150 ml
Renewal of Test Solution	daily	daily
Age of Test Organism	48 hr nauplii	3rd instar
Number of Organisms per Test Chamber	20	10
Number of Replicates per Concentration: range finder (acute test)	3(5)	3(5)
Number of Organisms per Concentration: range finder (acute test)	60(100)	30(50)
Feeding	Daily (see text)	Daily (see text)
Endpoint	mortality (LC50) and immobility (EC50)	mortality (LC50) and immobility (EC50)
Test Acceptability	≥ 90% control survivorship	≥ 90% control survivorship

Table 3. Summary of acute testing methods.

Time (hours)	0	24		48		72		96
Exposure Solutions	initial	initial	final	initial	final	initial	final	final
Control	1	1	2	1	2	1	2	2
Brine Shrimp	1	1	1	1	1	1	1	1
Brine Shrimp	1	1	1	1	1	1	1	1
Both	1	1	2	1	2	1	2	2
Both	1	1	2	1	2	1	2	2
Both	1	1	2	1	2	1	2	2
Brine Flies	1	1	1	1	1	1	1	1
Brine Flies	1	1	1	1	1	1	1	1
Duplicate	1	1	1	1	1	1	1	1

Table 4. Minimum number of analytical samples for each toxicant tested (see text for details

Chronic Toxicity Test Plan of Work:

While conducting the acute testing portion of this project, we will develop a plan of work for chronic testing of the priority pollutants described above.

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Appendix 1

Bioassay Water For Great Salt Lake Bioassays

State of Utah
GARY R. HERBERT
Governor

Department of
Environmental Quality

Alan Matheson
Executive Director

M E M O R A N D U M

TO: Great Salt Lake Bioassay Team

FROM: Chris Bittner

DATE: April 23, 2015 (edited for inclusion in Work Plan 5/4/16)

PROJECT: Great Salt Lake Toxicity Bioassays for Brine Shrimp and Brine Flies

SUBJECT: Bioassay water for Great Salt Lake Toxicity Bioassays

Conclusions

After evaluating how well each media met the data quality objectives, none of the media can currently be concluded to be superior. The DWQ Round Robin medium was scored the highest but has not been tested with the test organisms and concerns remain regarding the long-term stability.

Data Quality Objectives

1. USEPA approval is required because the test results are intended to ultimately support the development and promulgation of numeric criteria. USEPA approval is not independent of the other data quality objectives (DQOs) because if the other DQOs are met, USEPA would likely approve the results for criteria development. However, the salinity of Great Salt Lake (GSL) is not specifically addressed by USEPA Guidance or Rules and unavoidable deviations from the existing guidance and rules are anticipated. These deviations must ultimately be acceptable to USEPA for criteria development.

2. The test medium must support the test organisms which at minimum are anticipated to include brine shrimp, brine flies, and green algae. If the test medium doesn't support the test organisms as defined by acceptable survival, growth, and reproduction in the negative controls, the results won't support the development of numeric criteria.

3. The test medium must have minimal potential confounders that either increase or decrease toxicity. The goal of having no potentially confounding issues is the ideal and meeting this

DQO is anticipated to limited to accepting the least amount of potential confounders. Confounders include factors such as ion balance, pH, presence of pollutants to be tested, dissolved organic carbon and hardness. Some of these factors are discussed in the context of DQO 5 because ideally, the medium mimics GSL's concentrations of these modifiers of toxicity.

4. The medium must be able to be replicated over time. Considerations for this DQO include that the source of the materials used for the medium should be stable over time and have documented quality control to ensure that any deviations can be identified and addressed if necessary. Based on the resources that are anticipated to be available for conducting bioassays and the number of existing pollutants, numeric criteria development for GSL is a long term project (e.g., 20+ years). Tests conducted 20 years should give the same results.

5. The DQO that the medium should be representative of Great Salt Lake is related to DQO 3 for minimal confounders affecting toxicity. The representativeness DQO is specifically specified because in a regulatory context, if bias is present, bias that overestimates toxicity is much more acceptable than bias that may underestimate toxicity. USEPA recommendations for toxicity testing media are intended to avoid underestimating toxicity for waters across the nation at the expense of potentially overestimating toxicity. To address this potential overestimation, effluent limits or potentially criteria can be modified on a site-specific basis using the Water Effects Ratio (WER). A WER is the ratio of toxicity between conducting bioassays using USEPA standard laboratory bioassay water and site water for dilution. The results may be the national criteria are either over- or under protective for the specific site. The GSL criteria are intended to be site-specific, so the bioassay medium should accurately reflect the toxicity, or lack thereof, of GSL waters obviating the need for WERs.

6. The medium should be stable over time. At minimum, the media must be stable for the test duration and ideally, the media would be stable over longer time frames. The stability of the medium may also affect the reproducibility of the toxicity testing.

7. The medium should be able to be replicated by any qualified laboratory. This DQO is similar to the other precision-related DQOs such as replication over time. The media composition must be sufficiently documented and the materials readily available to any qualified laboratory to meet this DQO.

8. Cost and convenience are the least important consideration but resource constraints are still an influential factor. When the scores for each DQO were summed, the cost and convenience score was not included.

Evaluation of Media

Several potential media were investigated:

- GSL Water (Brix et al., 2006)
- Barnes and Wurtsbaugh (2015)
- Belovsky
- DWQ Marinemix
- DWQ Round Robin

The advantages and disadvantages of each approach relative to the data quality objectives (DQOs) are summarized in the following text. Media that met, or were anticipated to potentially meet the minimum requirements are qualitatively scored on a scale of 1 to 10 for each DQO. Scores are summarized in Table 5.

Brix et al. 2006

Brix et al. (2006) used GSL water, artificial seawater, and the Bagshaw et al. (1986) media for conducting their bioassays. Although not documented in the paper, Bill Adams' (coauthor with Brix) recollection was that the GSL water was not filtered. When contacted, Mr. Adams opined that because of the quantities of water needed, filtering through a 0.45 µm filter wasn't a practical option. While a larger filter may not clog as fast, decanting may be just as efficient as filtering. Brix et al. (2006) diluted the GSL water with deionized water to the salinity of seawater. Brix et al. (2006) concluded that the toxicity of copper in GSL water was much less compared to artificial seawater or the media of Bagshaw et al. (1986). Neither the artificial seawater medium nor the Bagshaw medium were considered further because they do not appear representative of Gilbert Bay water with regards to toxicity.

The use of GSL water might be approvable by USEPA. At minimum, this will require addressing the existing contaminant concentrations assuming they are low enough to not significantly affect the test results. A score of 7 is assigned for USEPA approval because using GSL water would require a deviation from existing USEPA protocols.

GSL water is anticipated to support the test organisms under current lake conditions and is scored a 10 for this DQO.

GSL water has many potential confounders that could affect the toxicity results. Existing pollutant concentrations (further discussed in the Marinemix section) for some metals are known but data for the majority of organic priority pollutants are unavailable. GSL water may also have unidentified pathogens or introduce other undesirable organisms into the bioassays. The concentration of dissolved organic carbon, sulfate, and hardness are expected to decrease the toxicity of metals. However, these affects are reflective of actual site conditions and are not considered undesirable for criteria development specific and limited to GSL. GSL water is scored a 5 for this DQO because of existing pollutant concentrations.

The consistency of GSL water over time is uncertain. Lake salinity is known to fluctuate over time (see Figure 1) and a protocol to address these potential fluctuations would need to be developed if salinity is determined to significantly affect toxicity. For instance, the test protocol may require salinities of 11 to 13%. If GSL water was higher, deionized water could be used to lower the salinity to the target range. UGS reports that the major ion ratios have remained similar (Gwynn, 1998). However, future changes are possible because of for instance, changes by the mineral extraction industries that selectively remove some of the salts and are required to return the remainder to the Lake. At GSL salinities lower than 11%, additional salts would have to be added. Depending on the quantity of salt required, the ion ratios of GSL water may not be achievable because maintaining the ion ratios may result in precipitation. This is judged to have a small potential because much higher salinities exist in GSL. Pollutant concentrations in GSL may increase over time resulting in the water being unacceptable for criteria development at some time

in the future. Other factors affecting toxicity such as pH, dissolved organic carbon, and sulfate would have to be tracked and their impacts on toxicity accounted for. GSL water is scored a 5 because of the identified uncertainties.

GSL water is representative of current conditions in GSL and is scored a 10. GSL water has several physiochemical parameters that are known to decrease toxicity and accurately accounting for these effects is important. GSL water is scored a 10 because the potential changes over time were addressed by the preceding DQO.

GSL water is anticipated to be stable relative to the concentrations of ions. The potential for biologically mediated changes are unknown. Based on the difficulty in obtaining and shipping the water, GSL water would have to be stable over time to allow large quantities to be collected and stored. Protocols would need to be established that verify the stability of GSL water over time. GSL water is scored an 8 for this DQO.

GSL water can be replicated by any laboratory provided that protocols are developed for collecting and storing the water. The protocols should also establish the tolerance ranges for key parameters. GSL water is scored a 10 for this DQO.

GSL water is anticipated to be costly because of the logistical issues of collection presumably by boat from the more remote areas of the Lake and the cost of shipping large quantities of water. Storage of the water may be inconvenient and the collection of additional water may be restricted by factors such as weather and/or season. GSL water is scored a 4 for this DQO.

Barnes and Wurtsbaugh, 2015

Barnes and Wurtsbaugh (2015) prepared a medium with salinity concentrations ranging from 10 g/l to 275 g/l. The media were prepared using equal parts deionized and GSL water and an inorganic salt mix consisting of 84% Instant Ocean, 14% NaCl, and 3% K_2SO_4. Final salinities and major ion concentrations were measured.

The Barnes and Wurtsbaugh (2015) medium would require similar analyses as the GSL water because it includes GSL water to meet USEPA approval. In addition, Instant Ocean is not a currently approved artificial salt mix for conducting bioassays. Presumably, USEPA could approve the use of this mix provided that protocols were developed to document the contents of the final solutions. This media is scored a 7.

The Barnes and Wurtsbaugh (2015) medium was used successfully to conduct experiments on brine shrimp. The suitability of this media for culturing algae or brine flies is unknown resulting in a score of 8.

Because the Barnes and Wurtsbaugh (2015) medium contains GSL water, the same concerns regarding confounders applies but to a lesser degree because only ½ of the liquid portion of the media is GSL water. The Instant Ocean has trace concentrations of the same inorganic substances that will be tested for the bioassays. Figures 2 through 8 compare the concentrations of metals in Instant Ocean at 120 ppt versus GSL water when data were available for both media. Note that none of the media considered is either 100% Instant Ocean or 100% Marinemix. With the exception of the DWQ Marinemix medium, this assumption is not anticipated to significantly

overestimate the metals concentrations because metals were not measured in all materials by Barnes and Wurtsbaugh (2015) or Belovsky.

The Instant Ocean concentrations were scaled up from the data of Hovanec and Coshland (2002). Atkinson and Bingman (1999), the only source for Instant Ocean trace metals that was peer reviewed, reported much higher trace metal concentrations than Hovanec and Coshland (2002) whose concentrations are corroborated by the data reported by Marulla and O'Toole (2005). As noted by Hovanec and Coshland (2002), the ICP/MS analytical method that they employed is considered more reliable than the ICP used by Atkinson and Bingman (1999). The comparisons were subsequently based on the data from Hovanec and Coshland (2002). The GSL data were reported by Adams et al., (2015) or were based on 2 years of recent sampling by DWQ. The GSL data were not normalized to 120 ppt salinity. The figures also include the same data for Crystal Sea Bioassay Laboratory Formula Marinemix which was provided by the manufacturer.

The following observations are based on a qualitative analysis of the comparisons shown on Figures 2 through 8. Arsenic concentrations are higher in the lake than in the Marinemix (Figure 2). No data were found for the Instant Ocean arsenic concentrations. Cadmium concentrations in Instant Ocean are higher than Marinemix or GSL (Figure 3). Copper concentrations are similar between Marinemix, Instant Ocean (not detected), and GSL (Figure 4). Lead (Figure 5) and zinc (Figure 9) concentrations in Marinemix are higher than Instant Ocean or GSL. Mercury concentrations are similar between Marinemix and GSL but no data were available for Instant Ocean (Figure 6). Nickel concentrations in Instant Ocean are higher than Marinemix and GSL (Figure7). Selenium concentrations are higher in Marinemix than GSL but no data were available for Instant Ocean (Figure 8). The media mixtures using Instant Ocean and Marinemix were was assigned a score of 7.

The Barnes and Wurtsbaugh (2015) media can be replicated over time. Although the medium would have some of the same potential deficiencies as were discussed for GSL water, the salinity can be increased with the salt mixture which would negate the impacts of varying salinity in the lake. However if other parameters in the GSL water changed significantly, the media may not easily replicated over time. The media was assigned a score of 7.

The Barnes and Wurtsbaugh (2015) media is reasonably similar to GSL water with respect to major ion concentrations (Table 2). However, the media has about half Ca and Mg as GSL water and higher concentrations of Cl and SO_4. The dissolved organic carbon concentration is not reported but is likely lower than the 7 to 42 mg/l reported by Brix et al. (2006) and Wurtsbaugh and Jones (2012), respectively. This media was scored 6 primarily due to the uncertainties regarding organic carbon.

The Barnes and Wurtsbaugh (2015) medium is presumed to be stable based on the duration of the microcosm experiments conducted. The medium was assigned a score of 10. The medium could also be replicated by a qualified laboratory and was assigned a score of 10. For cost and convenience, the medium was assigned a score of 6 because compared to 100% GSL water, half as much water is needed.

Table 2 excerpted from Barnes and Wurtsbaugh (2015)

Table 1. Ionic weight proportions of the Gunnison Bay (328 g L^{-1}) and Gilbert Bay (110 g L^{-1}) of the Great Salt Lake (from Sturm 1980) and measured ionic composition of water from six of the salinity treatments in the microcosm experiment.

	Great Salt Lake		Microcosm Experiment (Nominal Salinities)					
Major Ions	Gunnison Bay (328 g L^{-1})	Gilbert Bay (110 g L^{-1})	10 g L^{-1}	50 g L^{-1}	100 g L^{-1}	150 g L^{-1}	200 g L^{-1}	250 g L^{-1}
Na^{+}	.320	.313	.350	.330	.307	.314	.313	.320
K^{+}	.026	.027	.017	.025	.024	.025	.024	.025
Mg^{+2}	.032	.035	.037	.020	.017	.016	.016	.016
Ca^{+2}	.001	.002	.004	.001	.001	.001	.001	.001
Cl^{-}	.554	.551	.502	.538	.571	.562	.563	.562
SO_4^{-2}	.067	.073	.090	.086	.080	.082	.083	.076

Belovsky

Belovsky has successfully used a 60:40 mix by volume of Morton Solar Salt and Instant Ocean to lab to culture brine shrimp and brine flies for the proposed bioassays. The Morton Solar Salt is commercial water softening salt extracted from GSL and the ion concentrations are as reported by the manufacturer. Both Belovsky and Wurtsbaugh use Instant Ocean in their media combined with either GSL water or salt extracted from GSL water and the following includes a specific discussion only where the scores or rationale differ.

Belovsky's medium has been successfully used to culture GSL algae, brine shrimp and brine flies and was assigned a score of 10. Belovsky's medium was assigned a score of 6 for potential confounders. No data for trace metals were available for the Morton Solar Salt and the score could increase or decrease if this data were obtained.

A comparison of the major ions in Belovsky's medium to GSL water is shown in Figure 10 for assessing the representativeness compared to GSL water. Belovsky's media has more NaCl and less K, SO_4, Ca, and Mg than GSL water. These concentrations were estimated assuming that 60:40 ratio by volume was equivalent to 60:40 by weight and the scores could change based on actual analytical or more refined nominal estimates.

Belovsky's medium is the least expensive medium considered and uses salts that are easily obtained and was scored 10 for cost and convenience.

DWQ Marinemix

A DWQ proposed medium consisting of Marinemix, NaCl and KCl in ratios of 84:13:2, respectively was attempted. This medium may have matched GSL water better than Barnes and Wurtsbaugh (2015) or Belovksy but when the medium was attempted, a precipitate formed and preliminary testing indicated lower survival of brine flies and brine shrimp, potentially in part due to reduced algal production. Jim from EPA unsuccessfully attempted to permanently dissolve the precipitate by reducing the pH. After consulting with the Marinemix manufacturer, Belvosky

reported that at salinities above approximately 5%, calcium would precipitate. This medium was abandoned because of the precipitate. The precipitation also suggests that the Marinemix (USPEPA approved) could not simply be substituted for Instant Ocean in Barnes and Wurtsbaugh's (2015) or Belovsky's media recipes.

DWQ Round Robin

DWQ initiated a laboratory round robin study to verify the analytical methods commonly used for analyzing the GSL samples. The initial matrix was formulated using reagent grade salts only and precipitates formed at salinities well below 12%. Different chemical forms of the salts were attempted and the medium shown in Table 3 did not exhibit a precipitate when anhydrous salts were used. Precipitates did form at salinities above 13% and the SO_4 was reduced by 50% as shown in Table 4 to prevent precipitation up to a salinity of 20%. The medium described in Table 4 was not considered further because the major ion concentrations deviate substantially from GSL. With salts of sufficient quality (e.g., laboratory grade), the medium in Table 3 would be approvable by USEPA. The primary unknown with this media is if the test organisms would tolerate it and if potential nutrient deficiencies exist. Because this is unknown, a score of 3 was assigned which could change if the organism were tested.

No potential confounders are identified and the medium was scored a 10. The medium would be replicable over time. The media is representative of GSL for the major ions but is lacking dissolved organic carbon, so was scored a 7. The potential addition of organic carbon could potentially change this score. The medium was stable over a couple of weeks but longer-term stability was not tested, so a score of 9 was assigned. The media can be replicated by any qualified laboratory and was scored a 10.

The salts are available from laboratory suppliers but the costs will be higher than e.g., Instant Ocean. Large term storage may require conditions to preserve the anhydrous condition of the salts resulting in a score of 6.

Table 3 DWQ Round Robin Media Matrix at 13% Salinity										
Salt	**Added (g/l)**	**[Na+]**	**[Mg++]**	**[K+]**	**[Ca++]**	**[H+]**	**[Cl-]**	**[SO4=]**	**[CO3=]**	**[OH-]**
CaCl2+2H2O	1.1				0.299944		0.530661			
MgCl2 (anhy)	8.1		2.06775				6.032335			
NaCl	106.56	41.91779					64.64221			
KCl	5.38			2.821532			2.558468			
CaSO4										
MgSO4 (anhy)	11.6		2.342456					9.258026		
Na2SO4										
K2SO4										
NaHSO4										
CaCO3										
MgCO3										
Na2CO3										
K2CO3										
NaHCO3	0.65	0.177883				0.007737			0.464379	
Ca(OH)2										
Mg(OH)2										
NaOH										
KOH										
Mass Fraction		0.317328	0.033245	0.021269	0.002261		0.556049	0.069789		
Target Conc. (g/l)	133.39	42	4.4	2.8	0.3	1E-08	74	9.3	0.43	0.000017
% of Target		**100.2**	**100.**	**100.8**	**100.0**		**99.7**	**99.5**	**108**	

Table 5 Qualitative Scores for Bioassay Media Considered				
Data Quality Objective	**GSL Water**	**Barnes and Wurtsbaugh (2015)**	**Belovsky**	**DWQ Round Robin**
USEPA Approval	7	7	7	10
Media supports test organisms	10	8	10	3
Media has minimal potential confounders affecting the toxicity	5	7	6	10
Media can be replicated over time	5	7	7	10
Media is representative of Great Salt Lake	10	6	6	7
Media is stable over test duration	8	10	10	9
Media can be replicated at any laboratory	10	10	10	10
Low Cost and Convenient	4	6	8	6
SUM without cost score	55	55	56	59

References

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Figures

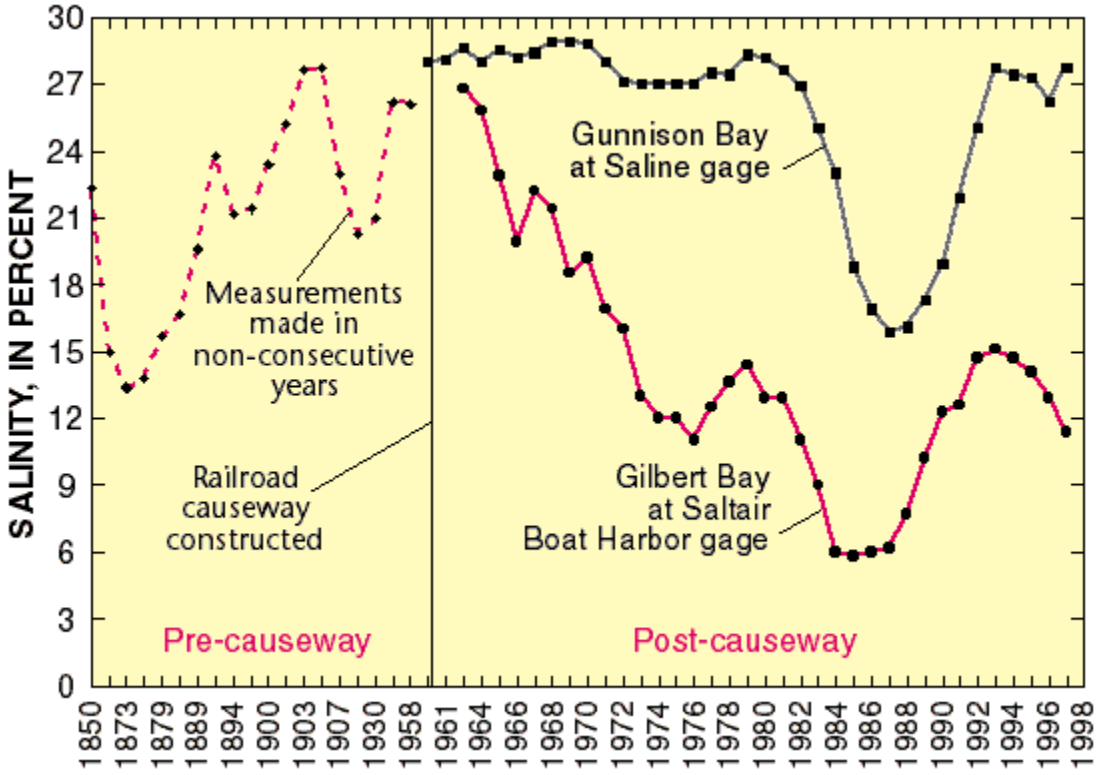


Figure 1. Great Salt Lake Salinity over time from USGS (http://ut.water.usgs.gov/greatsaltlake/salinity/)

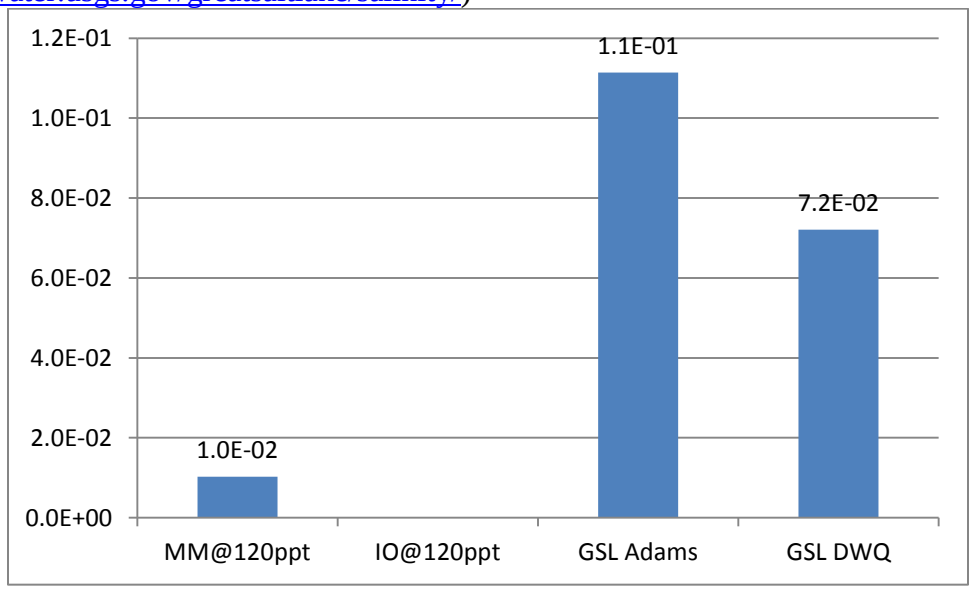


Figure 2. Comparison of mean arsenic concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data available for Instant Ocean (IO).

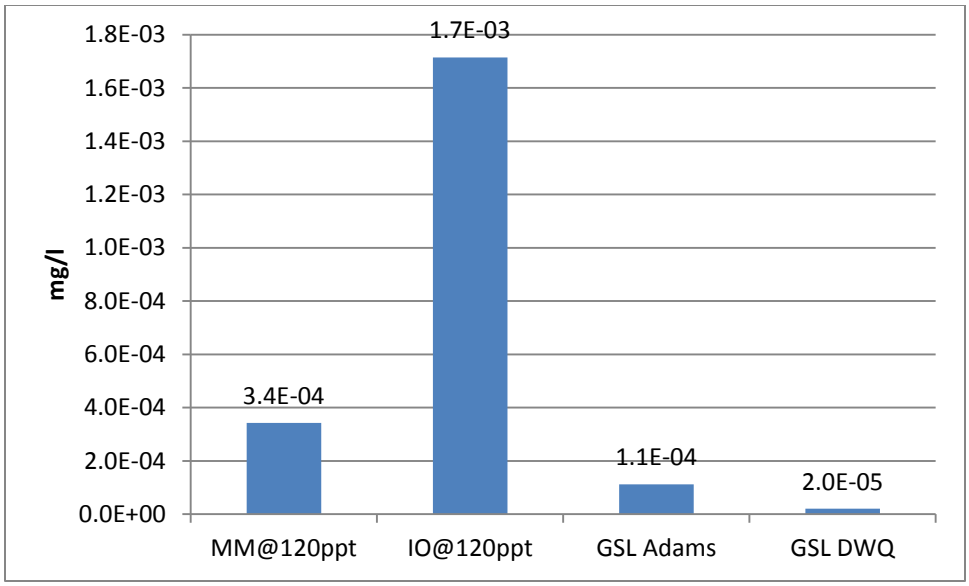


Figure 3. Comparison of mean cadmium concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality

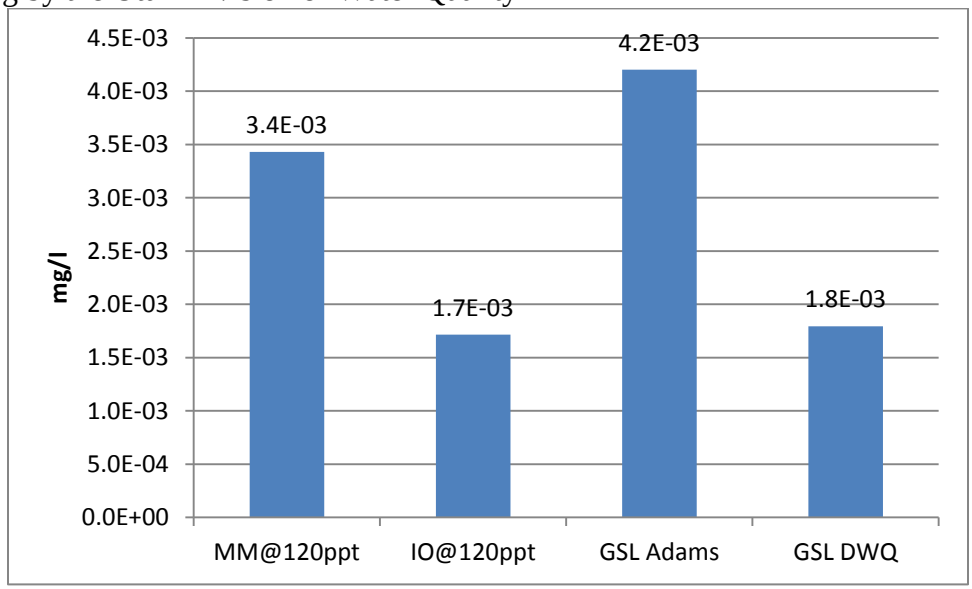


Figure 4. Comparison of mean copper concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Copper was nondetect for Instant Ocean.

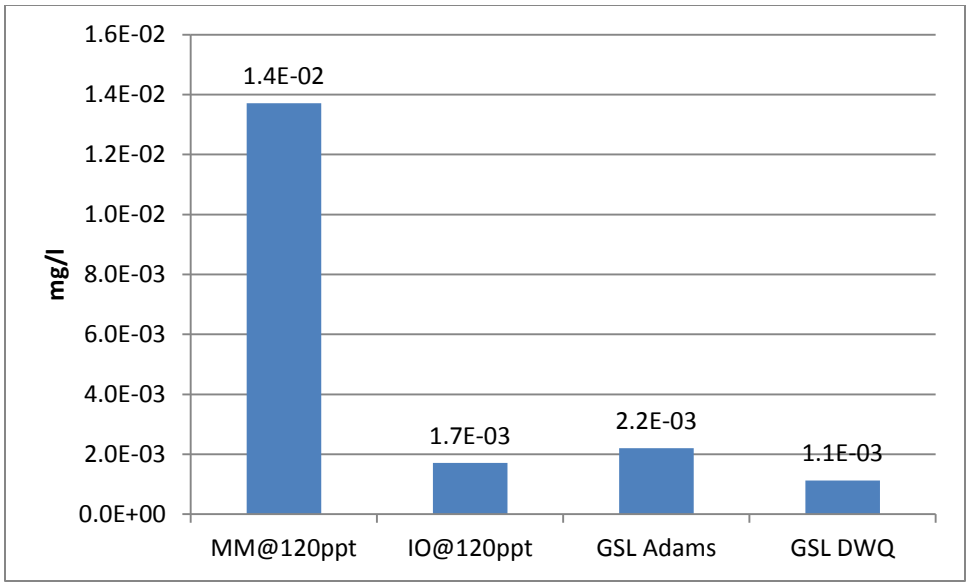


Figure 5. Comparison of mean lead concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Lead was nondetect for Instant Ocean.

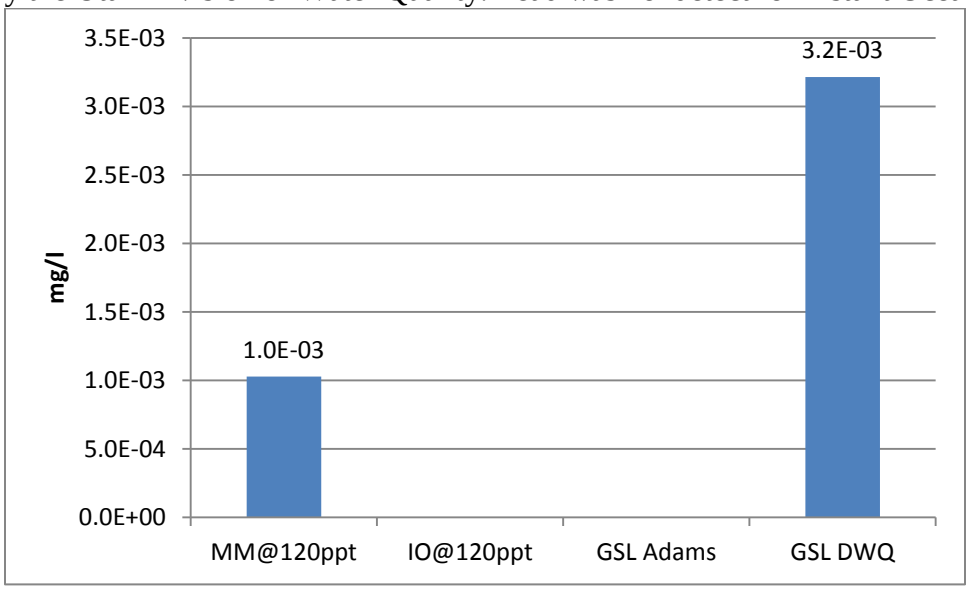


Figure 6. Comparison of mean lead concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data were available for Instant Ocean or GSL Adams.

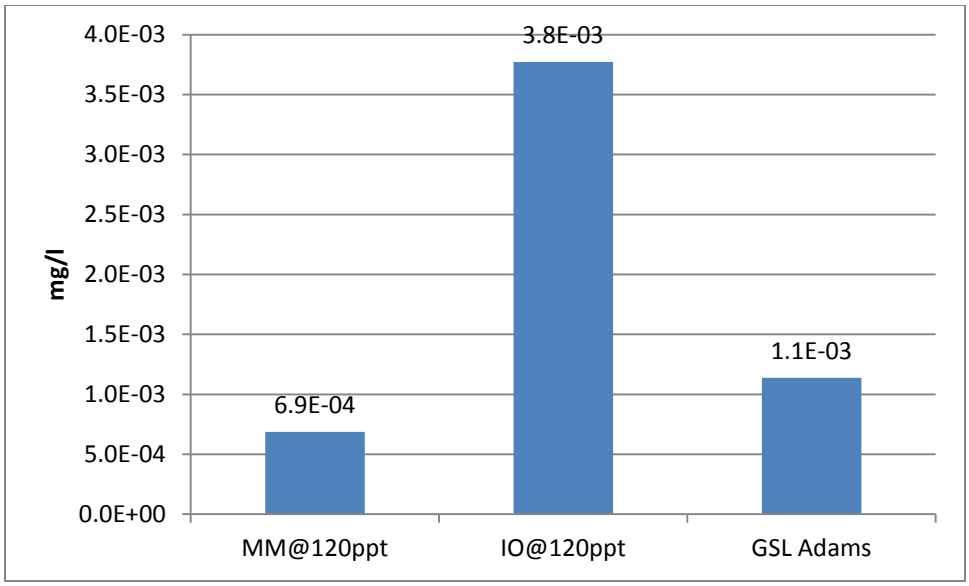


Figure 7. Comparison of mean nickel concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality

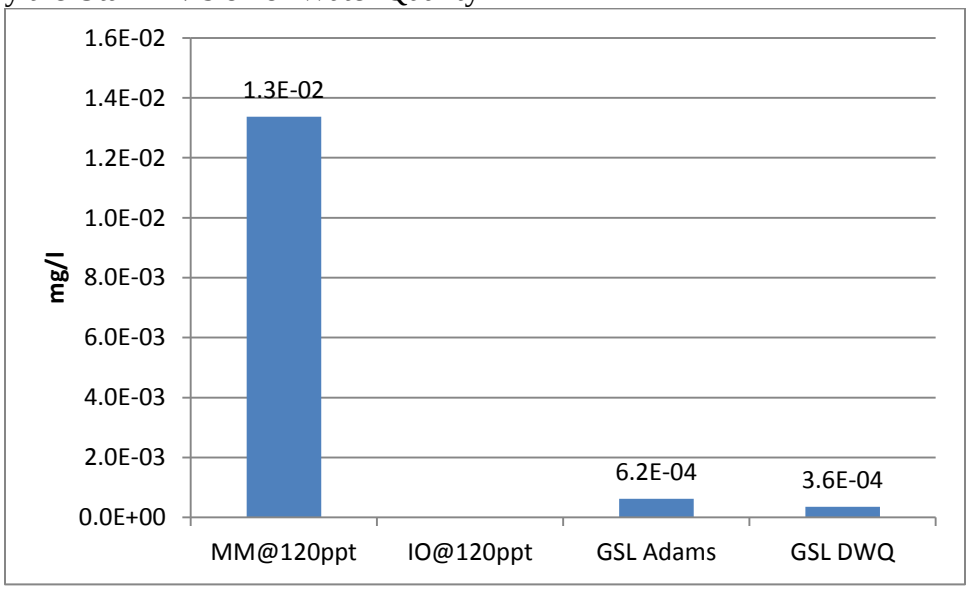


Figure 8. Comparison of mean selenium concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. No data were available for Instant Ocean.

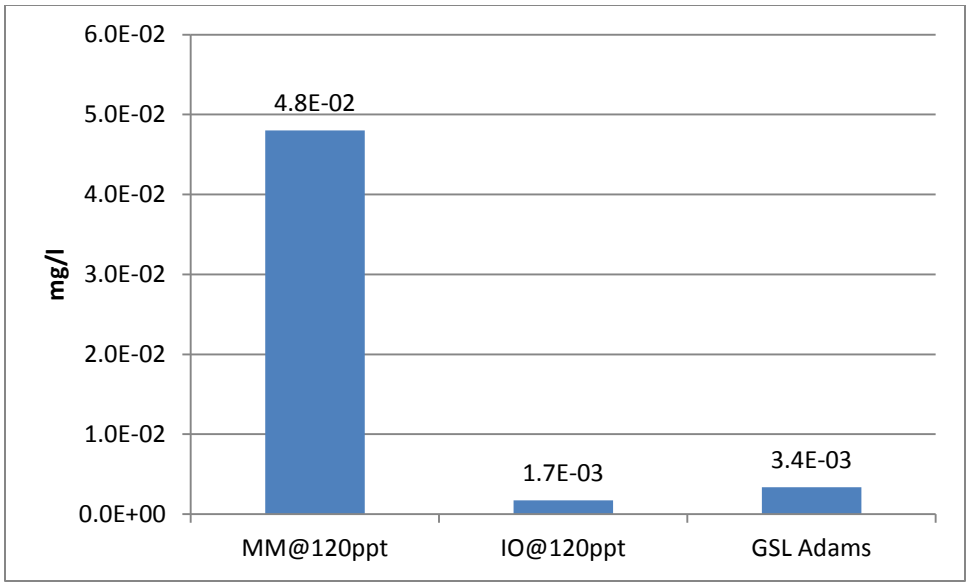


Figure 9. Comparison of mean zinc concentrations. MM=Marinemix, IO=Instant Ocean, GSL Adams=Great Salt Lake Adams et al.,2015, GSL DWQ= Great Salt Lake based on 2 years of sampling by the Utah Division of Water Quality. Zinc was nondetect for Instant Ocean.

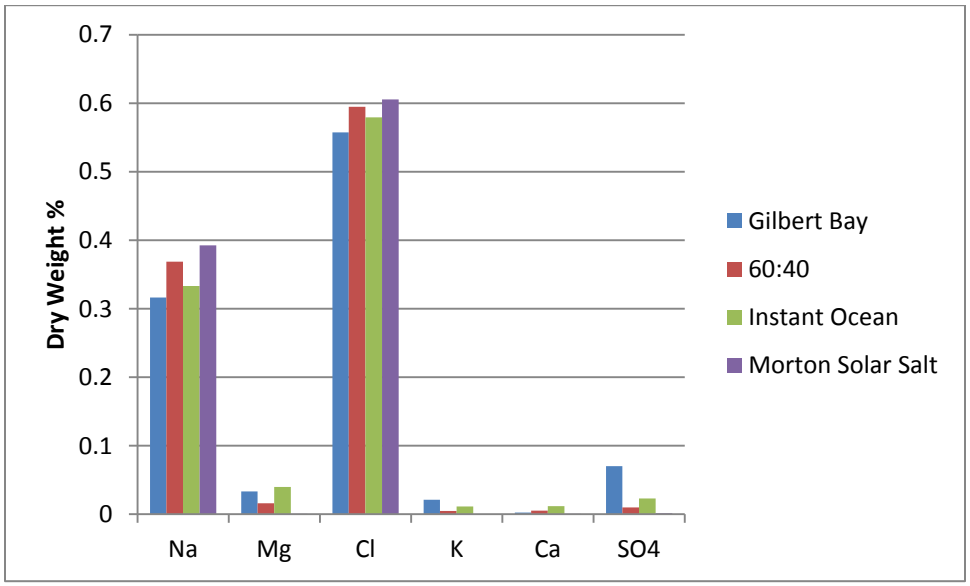


Figure 10. Comparison of major ions between Great Salt Lake (Gilbert Bay), a 60:40 Morton Solar Salt : Instant Ocean assuming volume is accurate surrogate for mass, Instant Ocean and Morton Solar Salt

APPENDIX 2

Results from Initial Range Finding for Copper and Brine Shrimp

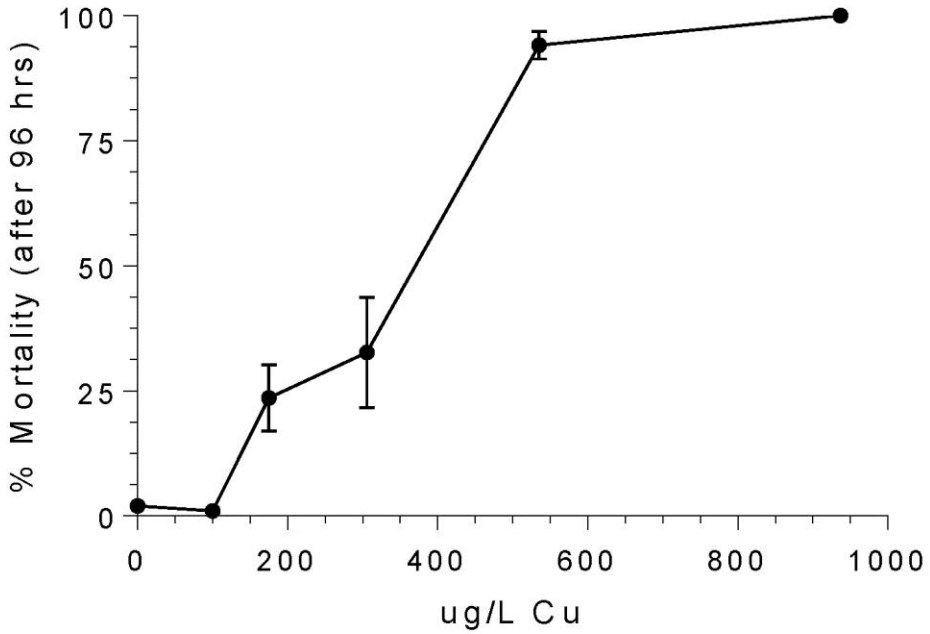
The pre-test water samples were collected after the water had been renewed, but prior to the addition of algae and brine shrimp each day as to hopefully ensure the correct copper concentrations were in solution.

The post-test water samples were collected each following day, prior to water renewal, as why those samples start at 24 hours. These samples were filtered in order to remove the algae.

The blanks were one replicate that was treated the same as all other replicates (water renewal each day and algae present), but it did not have brine shrimp in solution. Water samples were taken from these replicates after each 24 hours (post-test) to monitor the copper concentrations without brine shrimp in solution.

Ultra-pure nitric acid prior to shipment to the lab and were clear at the time of shipping.

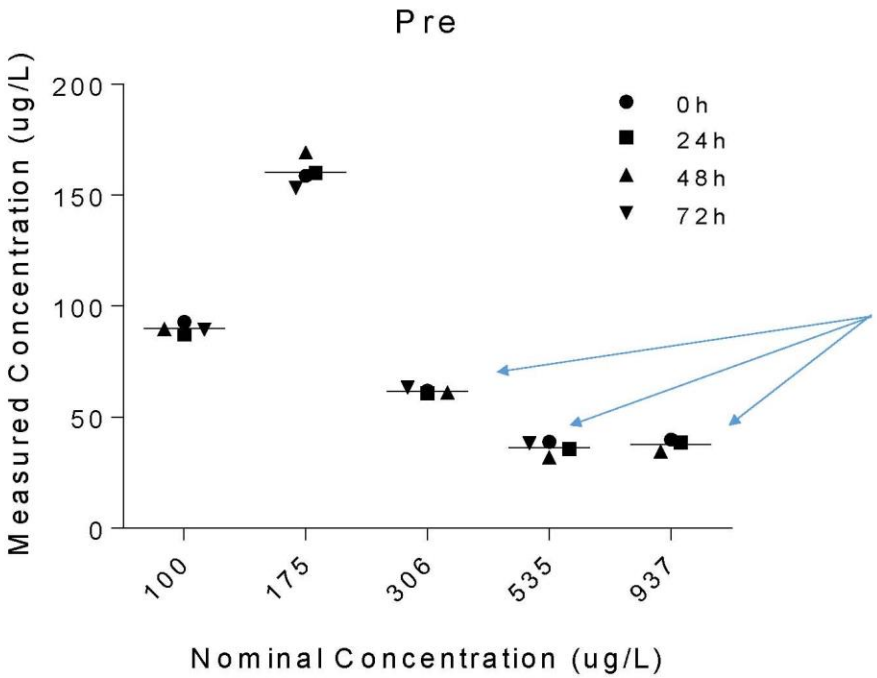
96-Hr Acute Copper Assay Results



Observations:

- Mortality increased with increased nominal concentrations of copper
- However, measured copper concentrations are not in agreement with nominal values…..see slides 2-5

Measured Copper Concentrations **Pre-test** for Each Day



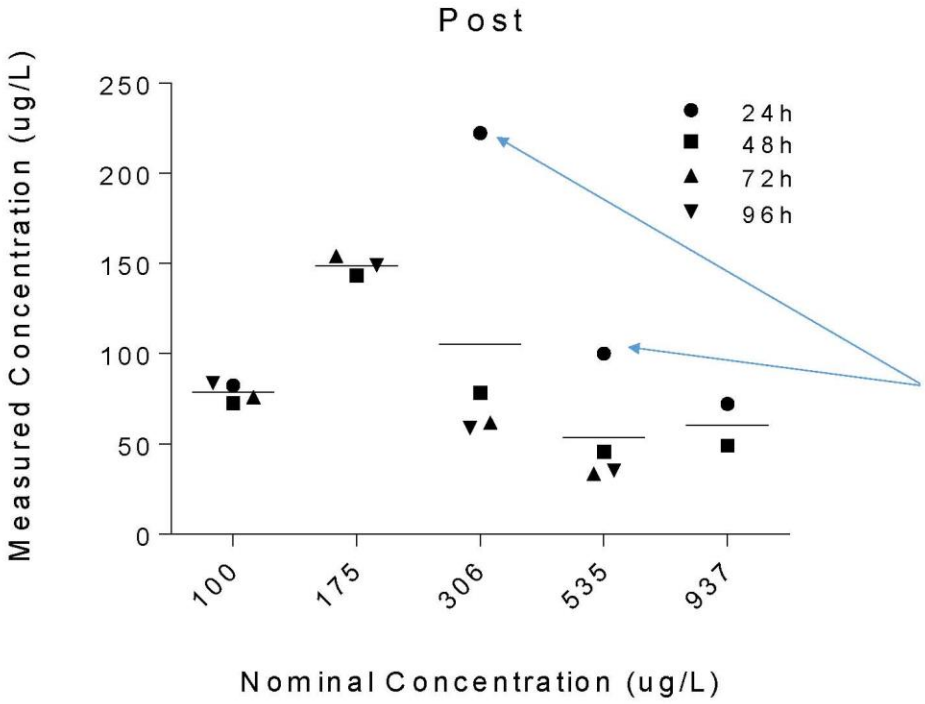
Observations:

- Background copper in control solutions varied from 5.3-9.6 ug/L
- Measured concentrations of copper at first 2 concentrations were acceptable
- The three highest concentrations had lower measured concentrations than expected

Possible Explanation: Precipitation and removal via filtration

*All samples were filtered via 0.2um syringe filters

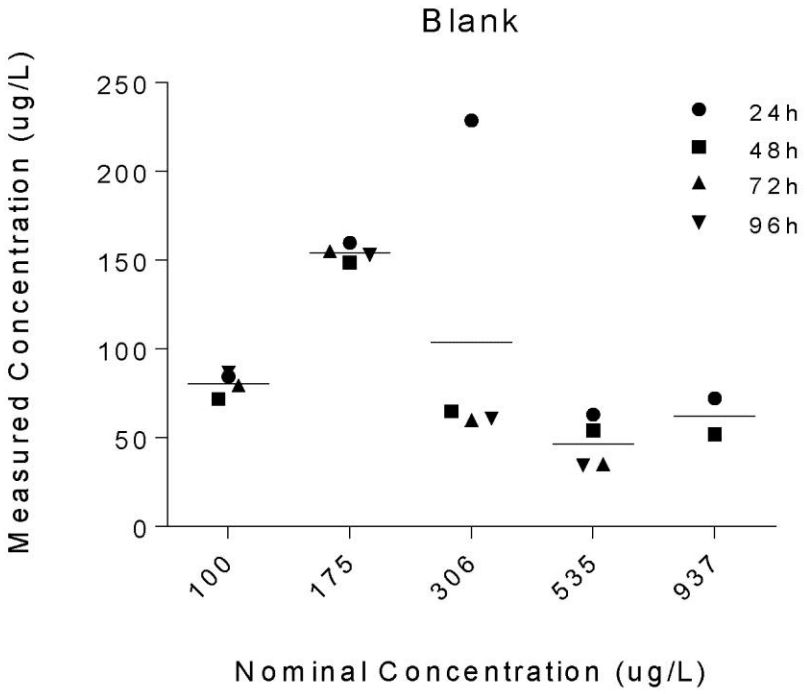
Measured Copper Concentrations **Post-test** for Each Day



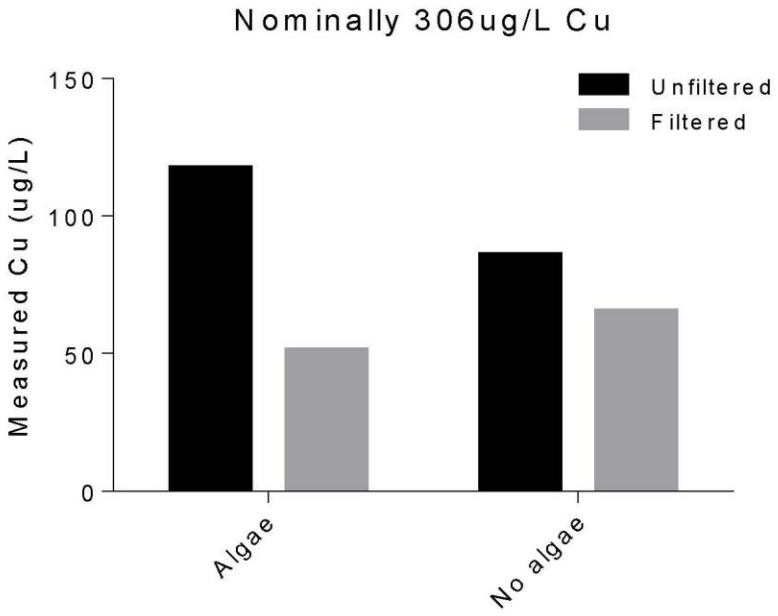
Observations:

- Similar trends in measured copper across the range of concentrations
- Reduction from pre-test concentrations, especially for 175 ug/L
  Possible explanation: Accumulation in algae (see slide 5)
- Higher concentration than pre test for certain measurements

Measured Copper Concentrations <u>Post-test (Blanks)</u> for Each Day



Filtered vs. Unfiltered Samples

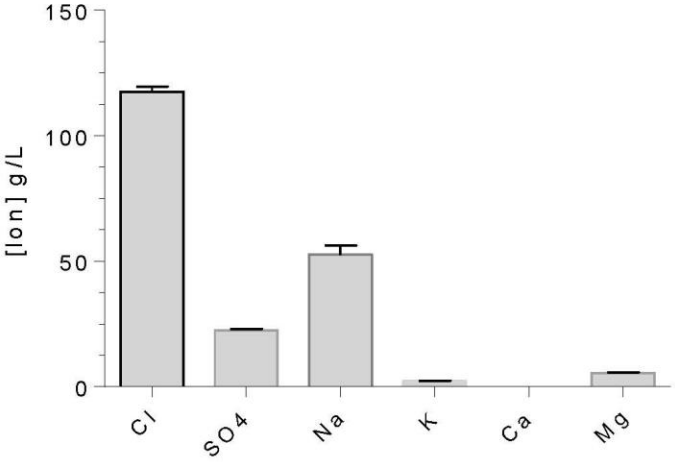


Observations:

- Filtering the sample via .2um has an effect on measured concentrations
- Copper appears to be associated with algae, either apically bound or accumulated
- Measured copper is lower than target concentration

Major Ion Concentrations

Ion	Average in lake (g/L)	Average measured (g/L)	Difference
Cl	74	116.5	42.5
SO4	9.3	22.8	13.5
Na	42	55.8	13.8
K	2.8	2.3	-0.5
Ca	0.3	0.34	0.04
Mg	4.4	5.3	0.9



A run of background metals will be important moving forward.
These initial samples were with a lower grade NaCl than what we are using now

APPENDIX B

ANALYTICAL CHEMISTRY INFORMATION

Analytical Data from Arsenic Study with Brine Fly Larvae (Ephydra cinerea) Study # 17001-474-029 June 11 - 15, 2018

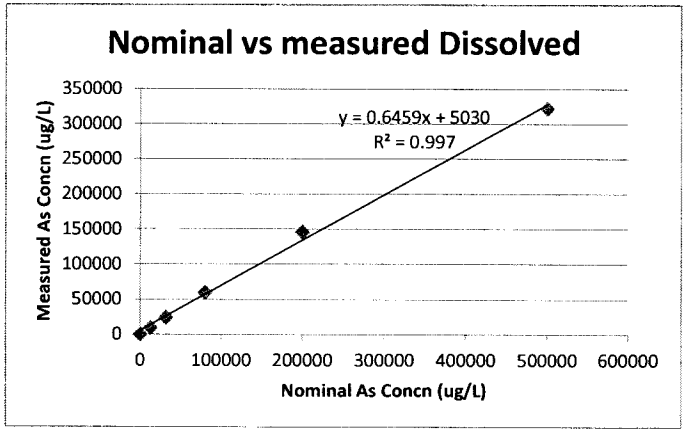
QA VZN 3/5/19
DRP 3/2/19

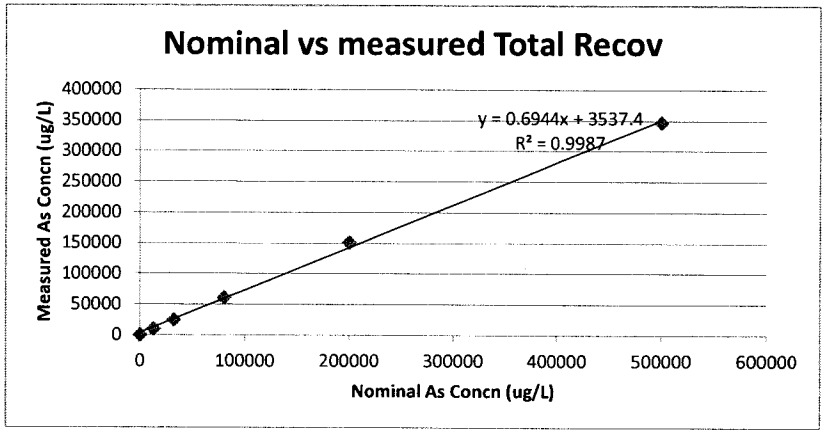
Final Analytical Data from ALS

Total Recoverable Nominal (ug/L)	Day 0 Time 0 (new)	48 h Time 1 (Old)	48 h Time 2 (New)	96 h Time 4 (Old)	Mean (ug/L)	Standard Deviation	Method Recovery Limit (ug/L)	Percent of Nominal
0	53 U	53 U	53 U	53 U	#DIV/0!	#DIV/0!	53	
12800	10000	8740	10600	9820	9790	775.37		76.484375
32000	25000	24300	25000	24600	24725	340.34		77.265625
80000	61200	62400	61200	60000	61200	979.8		76.5
200000	155000	155000	154000	141000	151250	6849.57		75.625
500000	369000	373000	361000	285000	347000	41633.32		69.4

Dissolved Nominal (ug/L)	Day 0 Time 0 (new)	48 h Time 1 (Old)	48 h Time 2 (New)	96 h Time 4 (Old)	Mean (ug/L)	Standard Deviation		Mean Diss/Mean TR	Initial Dissolved/ Initial TR
0	53 U	53 U	53 U	53 U	#DIV/0!	#DIV/0!	53		
12800	9640	8660	10200	9540	9510	636.76		74.296875	97.14	96.4
32000	24800	24000	24900	23300	24250	750.56		75.78125	98.08	99.2
80000	60100	61200	60200	58200	59925	1252.66		74.90625	97.92	98.2
200000	149000	152000	150000	135000	146500	7767.45		73.25	96.86	96.13
500000	370000	369000	353000	199000	322750	82866.86		64.55	93.01	100.27

MQ Blank 53 U





MSN 2/26/19

QA: DMP 3/1/19

Long list of metals - pre-definitive studies
RW#: 13060
date: Oct. 2017

	ug/L TR	ug/L Diss.
Antimony	1.0 U	1.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	1.82	0.65
Copper	0.29	0.35
Lead	0.271	0.304
Mercury	0.20 U	0.20 U
Nickel	6.88	4.29
Selenium	1.0 U	1.0 U
Silver	0.020 U	0.020 U
Thallium	0.053	0.160
Zinc	2.00	1.07

Long list of metals for arsenic definitive study
RW#: 13090
date: Nov. 2017

Brine Shrimp

	ug/L TR	ug/L Diss.
Antimony	2.0 U	2.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	0.44	0.47
Copper	0.21	0.36
Lead	0.043	0.150
Mercury	0.20 U	0.20 U
Nickel	14.6	14.1
Selenium	1.0 U	1.1
Silver	0.020 U	0.020 U
Thallium	0.020 U	0.038
Zinc	2.74	7.63

Long list of metals for lead definitive study - Brine Shrimp
RW#: 13175
date: Feb. 2018

No Food SP1.0hN-T/D(NF)

	ug/L TR	ug/L Diss.
Antimony	1.0 U	1.0 U
Arsenic	0.50 U	0.50 U
Beryllium	0.020 U	0.020 U
Cadmium	0.020 U	0.020 U
Chromium	0.56	0.25
Copper	0.41	0.28
Lead	0.077	0.032
Mercury	0.20 U	0.20 U
Nickel	22.0	22.0
Selenium	1.0 U	1.0 U
Silver	0.044	0.020 U
Thallium	0.102	0.022
Zinc	9.68	6.21

Food SP1.0hN-T/D(F)

	ug/L TR
Antimony	1.0 U
Arsenic	0.50 U
Beryllium	0.020 U
Cadmium	0.026
Chromium	37.2
Copper	4.93
Lead	0.898
Mercury	--- *
Nickel	23.4
Selenium	--- *
Silver	0.208
Thallium	0.465
Zinc	52.6

U = < MRL / MDL

*-insufficient volume for Hg and Se

APPENDIX C

BIOLOGICAL TEST DATA

QA Form No. 051
Revision 5
Effective 02/14
QA MW 3/5/19

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute

Project Number: 17001-474- 029

Test Substance: Na2HASO4

Species: *Ephydra cinerea*

Dilution Water: 120 ppt rGSL

Organism Lot or Batch Number: 18-015

Concurrent Control Water: NA

Age: 3rd Inst (~ 3rd instar

Supplier: Notre Dame

Date and Time Test Began: 6/11/18 @ 1900

Date and Time Test Ended: 6/15/18 @ 1900

Protocol Number:

Investigator(s): DAP/MW/AV/DM

Background Information

pH control?: Yes **No**

Type of Test: Static - Renewal

If yes, give % CO_2: N/A

Test Temperature: 20 ± 1 °C

Env. Chmbr/**Bath #**: 1

Test Chmbrs: 384 ml cups

Photoperiod: 16 h light : 8 h dark

Light Intensity: 50 - 100 ft-c.

Test Solution Vol.: 150 ml

Replicates per Treatment: 5

Length of Test: 96 h

Organisms per Replicate: 10

Type of Food and Quantity per Chamber: see food sheet

Feeding Frequency: On days 0 and 2

Test Substance Characterization Parameters and Frequency:

Hardness: Initiation

Alkalinity: Initiation

NH_3: Initiation

TRC: Initiation

pH: Daily*

Conductivity: Daily* & Termination

Salinity: Initiation

Test Concentrations (Mass:Volume): 0 (Control), 12.8, 32, 80, 200, and 500 mg/L As (nominal)

Agency Summary Sheet(s)?: NA

Reference Toxicant Data: Test Dates: N/A to N/A LC_{50}: N/A

Hist. 95% Control Limits: N/A to N/A Method for Determining Ref. Tox. Value: N/A

Special Procedures and Considerations:

* Measure chemistries at test termination or when 100% mortality is observed in a treatment.

Study Director Initials: DAP Date: 6/11/18

QA LWS 3/5/19

TEST SUBSTANCE USAGE LOG

Project Number: 17001-474- 029

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	C17-021			
Test Substance Stock Solution Preparation Date	6/11/18			
Sample Type	N/A			
Date Test Substance Received	N/A			
Dilution Water Number RW# or TRE#, circle one	rGSL # 13316	rGSL # 13340		
Concurrent Control Water RW#	N/A	N/A		
Date(s) Used	6/11/18	6/13/18		

Preparation of Test Solutions

Test Substance Conc. (mg/L As Nominal)	Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)		Test Substance Volume (ml)	Dilution Water Volume (ml)	Total Volume (ml)
0				see As spiking sheet			
12.8				"			
32.0				"			
80				"			
200				"			
500				"			
Total	0	0	0				

Initials / Date	6/11/18 DSP		
Initials / Date	6/13/18 DSP		
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			
Initials / Date			

QA NZN 3/5/19

ACUTE BIOLOGICAL DATA

Project Number: 17001-474-029

Test Species: ***Ephydra cinerea***

(mg/L) Conc.	Test Replicate	Number of Surviving Organisms: 0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	Remarks
0	A	10		10		9	90%
	B	10		10		9△	△1 NF
	C	10		10		10	
	D	10		10		10	
	E	10		9		7	
12.8	A	10		8		6	~~72%~~
	B	10		9		8	70.54% ⑤
	C	10		8		6+	+1 NF
	D	① ~~10~~ 11		① ~~11~~ 9		8	
	E	10		9		8	
32.0	A	10		9		9	72%
	B	10		② ~~9~~ 10		8	
	C	10		9		④ ~~7~~ 8	
	D	10		~~9~~ 6		3	
	E	10		10		8△	△1 NF
80	A	10		8		8	74%
	B	10		9		5	
	C	10		10		~~9~~ 10 ③	
	D	10		10		8	
	E	10		10		6	
200	A	10		4		1△	△2 NF 18%
	B	10		3		1	
	C	10		5		2△	△1 NF
	D	10		3		2	
	E	10		5		3+	+1 NF
500	A	10		0		—	0%
	B	10		2		0	
	C	10		1		0	
	D	10		1+		0	+1 org NF
	E	10		0		—	
	Date:	6/11/18		6/13/18		6/15/18	
	Time:	1900		1730		~~1545~~ ④ 1900	
	Initials:	DAP		[illegible]		[illegible]	

① Dep 6/13/18 E ② [illegible] 6/13/18 E ③ a 6/15/18 E ④ Dep 6/15/18 E ⑤ [illegible] 7/17/18 E

CDA MZW 3/5/19

ACUTE CHEMICAL DATA

Project Number: 17001-474-029

Test Species: ***Ephydra cinerea***

	NEW				OLD				Meter # (All Conc.)				
(mg/L)	Day 0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
Conc.: 0					Rep.	Rep. A	Rep.	Rep. B					
pH	7.8		8.9			7.7		8.1	26		FM26		FM26
D.O. (mg/L) ②	7 4.78		4.58		4.76	③ 4.58		4.29	LDO		LDO		LDO
Temp. (°C)	22		22		22	22		22	L-13		L-13		L-13
Cond. (µS/cm)	~~140100~~		131400			③ ~~131,900~~		129100	15		15		15
Hard. (mg/L) ①	139100								Titration				
Alk. (mg/L)									Titration				
TRC (mg/L)													
NH3 (mg/L)	122												
Salinity (ppt) ②	~~123~~		122						1				
Conc.: 12.8													
pH	7.8		8.9			7.7		8.1					
D.O. (mg/L)	4.79		4.66		4.65	③ ~~4.66~~		4.24					
Temp. (°C)	22		22			22		22					
Cond. (µS/cm) ①	~~140000~~		129400			③ ~~130,000~~		127800					
Hard. (mg/L)	139800												
Alk. (mg/L)													
TRC (mg/L)													
NH_3 (mg/L)													
Conc.: 32.0													
pH	7.8		8.8			7.7		8.1					
D.O. (mg/L)	4.79		4.74		4.63	③ ~~4.74~~		4.27					
Temp. (°C)	22		22			22		21					
Cond. (µS/cm)	135200		130400			③ ~~130400~~		128100					
Conc.: 80													
pH	7.7		8.8			7.7		8.1					
D.O. (mg/L)	4.75		4.72			4.63		4.25					
Temp. (°C)	22		22			22		22					
Cond. (µS/cm)	134500		130,100					127900					
Date:	6/11/18		6/13/18			6/13/18		6/15/18					
Time:	1915		1545			1545		1740					
Initials:	DAP		MW			MW		DAP MW					

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log, QA Form No. 084

① DAP 6/11/18 FC ② DAP 6/11/18 WP ③ MW 6/13/18 WP

QA NBW 3/5/19

ACUTE CHEMICAL DATA

Project Number: 17001-474-029

Test Species: ***Ephydra cinerea***

	NEW				OLD				Meter # (All Conc.)				
(mg/L)	Day 0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
Conc.:					Rep.	Rep. A	Rep.	Rep. B					
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.:													
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.:													
pH													
D.O. (mg/L)													
Temp. (°C)													
Cond. (µS/cm)													
Conc.: 200													
pH	7.7		8.6			7.7		8.0					
D.O. (mg/L)	4.71		4.73			4.62		4.27					
Temp. (°C)	21		22		~~27~~ (1)	22		21					
Cond. (µS/cm)	133400		141,200					127500					
Conc.: 500													
pH	7.7		8.4			7.7		7.5					
D.O. (mg/L)	4.70		4.80			4.53		4.37					
Temp. (°C)	21		23			22		21					
Cond. (µS/cm)	132900		131,700			130,800		128300					
Hard. (mg/L)													
Alk. (mg/L)													
TRC (mg/L)													
NH3 (mg/L)													
Salinity (ppt)													
Date:	6/11/18		6/13/18			6/13/18		6/15/18					
Time:	1915		1545			1545		1740					
Initials:	DSP		MW			MW		DSP					

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log, QA Form No. 084

(1) MW 6/13/18, Wf

QA Form No. 055
Revision 3
Effective 02/14
QA NBN 3/5/19

DAILY TOXICITY TEST LOG

Project Number:	17001-474- 029
Test Species:	***Ephydra cinerea***

General Comments	Measured salinity of rGSL water: 122 ppt Random Chart Sec 4 Hawthorn Thermometer #: L-29	Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: 1300 Food Added at: 1530 Test Organisms Added at: 1900	Algae on Filters added to test chambers ~ 1530	DAP 6/11/18
Test Day 1	Real Time Temp.= 19 °C Range = 19 - 21 °C	None	DAP 6/13/18
Test Day 2	Real Time Temp.= 20 °C Range = 19 - 20 °C	Algae on filters added to test chambers ~ 1400	DAP 6/13/18
Test Day 3	Real Time Temp.= 20 °C Range = 20 - 20 °C	None	DAP 6/14/18
Test Day 4	Real Time Temp.= 21 °C Range = 20 - 21 °C		DAP 6/15/18
Test Day 5			
Test Day 6			
Test Day 7			
Test Day 8			

Brine fly Definitive test 2nd Test Initiated: **11-Jun-18** DAP 6/11/18 QA NW 3/5/19

17-Apr-18 **Brine fly Definitive test**

Primary stock @ 8,000.00 mg/L As = 33.3166 g Na2HAsO4 * 7H2O / L H2O

Volume per treatment (L) 1

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)
6	500,000	62.50	1.00
5	200,000	25.00	1.00
4	80,000	10.00	1.00
3	32,000	4.00	1.00
2	12,800	1.60	1.00
1	0	0.00	1.00
Total		103.10	6.00

0.4 dilution series

6.3% % spike of vol

Day 1 volumes & Stock Additions

TOTAL VOL
12.0 L
3.43 gallons

Conc. at Test Renewal

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)
6	500,000	62.50	1.00
5	200,000	25.00	1.00
4	80,000	10.00	1.00
3	32,000	4.00	1.00
2	12,800	1.60	1.00
1	0	0.00	1.00
Total		103.10	6.0

Analytical Volume: 100 ml per concentration (plus duplicates) at test initiation, new dissolved
100 ml per concentration (plus duplicates) at test renewal, old dissolved
100 ml per concentration (plus duplicates) at test renewal, old total recoverable

Brine fly Definitive test 2nd Test Initiated: **11-Jun-18**

DAP 6/13/18 QA com 7/5/19

17-Apr-18 **Brine fly Definitive test**

Primary stock @ 8,000.00 mg/L As = 33.3166 g Na2HAsO4 * 7H2O / L H2O

Volume per treatment (L) **0.8**

0.4 dilution series

6.3% % spike of vol

Trtmnt	Conc. ug/L	Stock **(ml)**	Total Vol(L)
6	500,000	50.00	0.80
5	200,000	20.00	0.80
4	80,000	8.00	0.80
3	32,000	3.20	0.80
2	12,800	1.28	0.80
1	0	0.00	0.80
Total		82.48	4.80

TOTAL VOL
9.6 L
2.74 gallons

Conc. at Test Renewal

Trtmnt	Conc. ug/L	Stock (ml)	Total Vol(L)
6	500,000	50.00	0.80
5	200,000	20.00	0.80
4	80,000	8.00	0.80
3	32,000	3.20	0.80
2	12,800	1.28	0.80
1	0	0.00	0.80
Total		82.48	4.8

Day 2 volumes + Stock Additions

Analytical Volume: 100 ml per concentration (plus duplicates) at test initiation, new dissolved
100 ml per concentration (plus duplicates) at test renewal, old dissolved
100 ml per concentration (plus duplicates) at test renewal, old total recoverable

Random Chart for As Brine Fly definitive study

2nd Test (w. 5 reps + 10 organisms / Rep) 3/5/19

Initiated on 6/11/18

0.040609	P1-4	1-D		A
0.052594	P3-1	3-A		B
0.058357	P2-5	2-E		C
0.064117	P5-1	5-A		D
0.111139	P4-2	4-B		E
0.155116	P2-2	2-B	12.8	A
0.180092	P6-4	6-D		B
0.214803	P4-5	4-E		C
0.241688	P1-1	1-A		D
0.258089	P6-5	6-E		E
0.260786	P3-5	3-E	32	A
0.316961	P1-5	1-E		B
0.331968	P3-2	3-B		C
0.348368	P1-2	1-B		D
0.375865	P5-5	5-E		E
0.385366	P4-1	4-A	80	A
0.387737	P6-2	6-B		B
0.399265	P2-4	2-D		C
0.462034	P2-3	2-C		D
0.46881	P2-1	2-A		E
0.474399	P5-3	5-C	200	A
0.490044	P5-4	5-D		B
0.505853	P6-1	6-A		C
0.658912	P6-3	6-C		D
0.669352	P3-4	3-D		E
0.690513	P4-3	4-C	500	A
0.894144	P4-4	4-D		B
0.90844	P1-3	1-C		C
0.953544	P5-2	5-B		D
0.975395	P3-3	3-C		E

CETIS Analytical Report

Ephydra cinerea ① TR As

Report Date: 18 Feb-19 13:58 (p 1 of 3)
Test Code: 474-029TR | 03-9551-2273

~~Fathead Minnow~~ 96-h Acute Survival Test

TRE Environmental Strategies, LLC

Analysis ID:	05-3854-2293	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Feb-19 13:58	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	13-1139-2997	**Test Type:**	Survival (96h)	**Analyst:**	Lab Tech
Start Date:	11 Jun-18 19:00	**Protocol:**	EPA/821/R-02-012 (2002) ①	**Diluent:**	~~Mod-Hard Synthetic Water~~ rGSL
Ending Date:	15 Jun-18 19:00	**Species:**	~~Pimephales promelas~~ Ephydra cinerea	**Brine:**	Not Applicable ①
Duration:	96h	**Source:**	~~In-House Culture~~ Notre Dame	**Age:**	3rd Instar
Sample ID:	10-9833-0334	**Code:**	417730DE	**Client:**	Confidential
Sample Date:	11 Jun-18 13:00	**Material:**	~~Manganese Chloride~~ Arsenic	**Project:**	Special Studies
Receive Date:	11 Jun-18 15:30	**Source:**	Research ① as Sodium Arsenate		
Sample Age:	6h (20 °C)	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.1	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
15	-132.8	272.5	275.8	5.067	0.1522	0.7489	1.539	3.009	0.2302	Non-Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	65530	5057	95610
LC10	74430	8438	103200
LC15	81100	11910	108700
LC20	86840	15650	113400
LC25	92070	19770	117600
LC40	106700	35480	129600
LC50	116600	50110	138300

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.9	0.9 - NL	Yes	Passes Acceptability Criteria

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.2272	0.03305	0.1624	0.2919	6.873	<0.0001	Significant Parameter
Slope	6.57	2.524	1.623	11.52	2.603	0.0148	Significant Parameter
Intercept	-33.29	13.05	-58.87	-7.707	-2.55	0.0167	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	116.1937	116.1937	1	88.5	<0.0001	Significant
Lack of Fit	5.717689	1.905896	3	1.539	0.2302	Non-Significant
Pure Error	29.73099	1.238791	24			
Residual	35.44868	1.312914	27			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	35.45	40.11	0.1279	Non-Significant Heterogenity
	Likelihood Ratio GOF	39.25	40.11	0.0601	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	1.028	2.773	0.4309	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.921	0.9303	0.0285	Non-normal Distribution
	Anderson-Darling A2 Normality	0.8424	2.492	0.0299	Non-normal Distribution

① DSP 3/18/19 CF

CETIS Analytical Report

Ephydra cinerea TRAs

Report Date: 18 Feb-19 13:58 (p 2 of 3)
Test Code: 474-029TR | 03-9551-2273

① ~~Fathead Minnow~~ 96-h Acute Survival Test

TRE Environmental Strategies, LLC

Analysis ID:	05-3854-2293	**Endpoint:**	96h Survival Rate
Analyzed:	18 Feb-19 13:58	**Analysis:**	Linear Regression (MLE)
CETIS Version:	CETISv1.8.7		
Official Results:	Yes		

96h Survival Rate Summary

			Calculated Variate(A/B)								
① C-~~mg~~µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	5	0.9	0.7	1	0.05477	0.1225	13.6%	0.0%	45	50
9790		5	0.7055	0.6	0.8	0.04505	0.1007	14.3%	21.6%	36	51
24725		5	0.72	0.3	0.9	0.1068	0.2387	33.2%	20.0%	36	50
61200		5	0.74	0.5	1	0.08718	0.1949	26.3%	17.8%	37	50
151250		5	0.18	0.1	0.3	0.03742	0.08367	46.5%	80.0%	9	50
347000		5	0	0	0	0	0		100.0%	0	50

96h Survival Rate Detail

① C-~~mg~~µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	0.9	0.9	1	1	0.7
9790		0.6	0.8	0.6	0.7273	0.8
24725		0.9	0.8	0.8	0.3	0.8
61200		0.8	0.5	1	0.8	0.6
151250		0.1	0.1	0.2	0.2	0.3
347000		0	0	0	0	0

96h Survival Rate Binomials

① C-~~mg~~µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	9/10	9/10	10/10	10/10	7/10
9790		6/10	8/10	6/10	8/11	8/10
24725		9/10	8/10	8/10	3/10	8/10
61200		8/10	5/10	10/10	8/10	6/10
151250		1/10	1/10	2/10	2/10	3/10
347000		0/10	0/10	0/10	0/10	0/10

① DSP 2/18/19 CF

CETIS Analytical Report

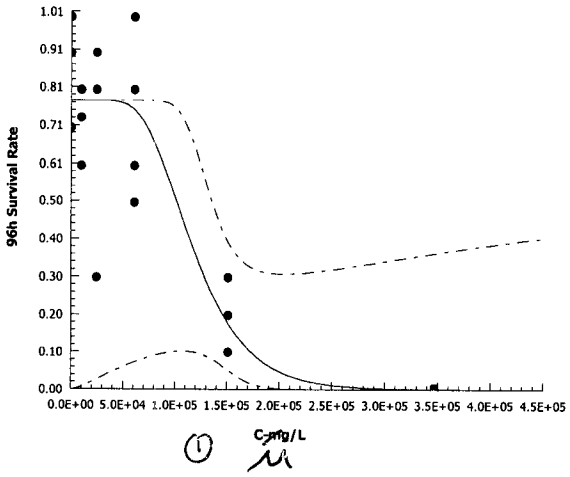
Ephydra cinerea

(1) ~~Fathead Minnow~~ 96-h Acute Survival Test

Report Date:	18 Feb-19 13:58 (p 3 of 3)
Test Code:	474-029TR | 03-9551-2273
	TRE Environmental Strategies, LLC

Analysis ID:	05-3854-2293	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Feb-19 13:58	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Graphics Log-Normal [NED=A+B*log(X)]



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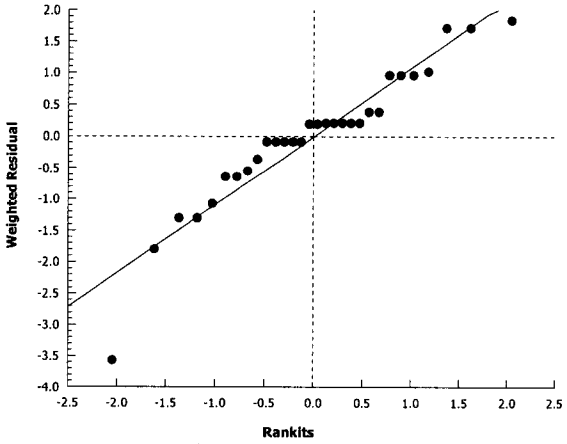

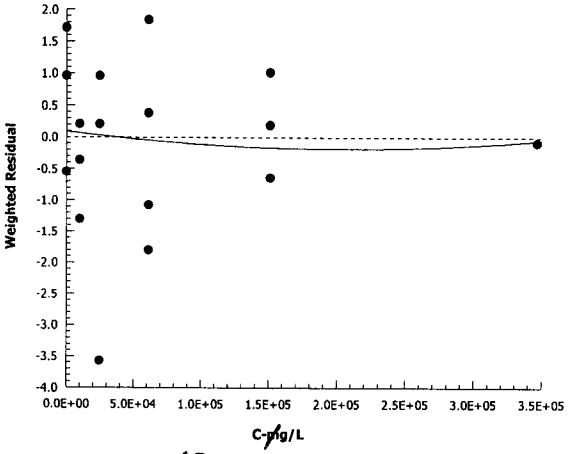

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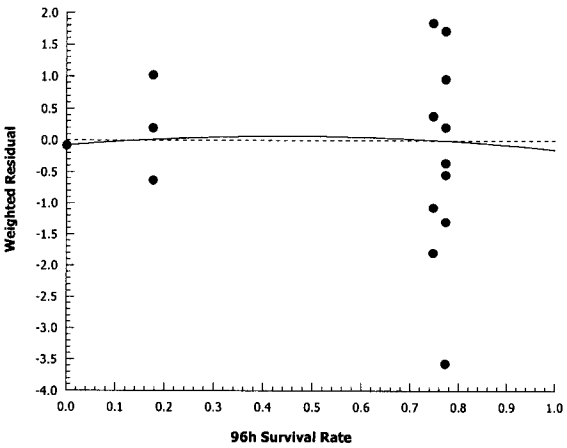


(1) DAP 7/18/19 CF

Analyst: JCP QA: DAP 7/18/19

CETIS Analytical Report

Ephydra cinerea (1) Dissolved As

Report Date: 18 Feb-19 14:00 (p 1 of 3)
Test Code: 474-029Dis | 13-0997-5831

~~Fathead Minnow~~ 96-h Acute Survival Test

TRE Environmental Strategies, LLC

Analysis ID:	09-2725-3283	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Feb-19 14:00	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	13-1139-2997	**Test Type:**	Survival (96h)	**Analyst:**	Lab Tech
Start Date:	11 Jun-18 19:00	**Protocol:**	EPA/821/R-02-012 (2002) (1)	**Diluent:**	~~Mod-Hard Synthetic Water~~ rGSL
Ending Date:	15 Jun-18 19:00	**Species:**	~~Pimephales promelas~~ Ephydra cinerea	**Brine:**	Not Applicable (1)
Duration:	96h	**Source:**	~~In House Culture~~ Notre Dame	**Age:**	3rd Instar
Sample ID:	18-3693-8102	**Code:**	6D7D7376	**Client:**	Confidential
Sample Date:	11 Jun-18 13:00	**Material:**	~~Manganese Chloride~~ Arsenic as	**Project:**	Special Studies
Receive Date:	11 Jun-18 15:30	**Source:**	Research (1) sodium arsenate		
Sample Age:	6h (20 °C)	**Station:**			

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.1	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
16	-132.8	272.6	275.8	5.056	0.1482	0.7488	1.543	3.009	0.2290	Non-Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	64860	4499	94170
LC10	73420	7585	101400
LC15	79820	10780	106600
LC20	85310	14250	111000
LC25	90310	18090	115000
LC40	104300	32870	126300
LC50	113700	46800	134400

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.9	0.9 - NL	Yes	Passes Acceptability Criteria

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.2276	0.0329	0.1631	0.2921	6.919	<0.0001	Significant Parameter
Slope	6.748	2.631	1.592	11.9	2.565	0.0162	Significant Parameter
Intercept	-34.12	13.58	-60.73	-7.507	-2.513	0.0182	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	116.1759	116.1759	1	88.44	<0.0001	Significant
Lack of Fit	5.735543	1.911848	3	1.543	0.2290	Non-Significant
Pure Error	29.73099	1.238791	24			
Residual	35.46654	1.313575	27			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	35.47	40.11	0.1274	Non-Significant Heterogenity
	Likelihood Ratio GOF	39.27	40.11	0.0598	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	1.03	2.773	0.4296	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9212	0.9303	0.0287	Non-normal Distribution
	Anderson-Darling A2 Normality	0.8421	2.492	0.0299	Non-normal Distribution

(1) Dsp 2/14/19 CF

3/2/19

Analyst: JP QA: KBW

CETIS Analytical Report

(1) Ephydra cinerea

Report Date: 18 Feb-19 14:00 (p 2 of 3)
Test Code: 474-029Dis | 13-0997-5831

~~Fathead Minnow~~ 96-h Acute Survival Test

TRE Environmental Strategies, LLC

Analysis ID: 09-2725-3283 Endpoint: 96h Survival Rate CETIS Version: CETISv1.8.7
Analyzed: 18 Feb-19 14:00 Analysis: Linear Regression (MLE) Official Results: Yes

96h Survival Rate Summary

(1)

C-µg/L	Control Type	Count	Calculated Variate(A/B) Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	5	0.9	0.7	1	0.05477	0.1225	13.6%	0.0%	45	50
9510		5	0.7055	0.6	0.8	0.04505	0.1007	14.3%	21.6%	36	51
24250		5	0.72	0.3	0.9	0.1068	0.2387	33.2%	20.0%	36	50
59925		5	0.74	0.5	1	0.08718	0.1949	26.3%	17.8%	37	50
146500		5	0.18	0.1	0.3	0.03742	0.08367	46.5%	80.0%	9	50
322750		5	0	0	0	0	0		100.0%	0	50

96h Survival Rate Detail

(1)

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	0.9	0.9	1	1	0.7
9510		0.6	0.8	0.6	0.7273	0.8
24250		0.9	0.8	0.8	0.3	0.8
59925		0.8	0.5	1	0.8	0.6
146500		0.1	0.1	0.2	0.2	0.3
322750		0	0	0	0	0

96h Survival Rate Binomials

(1)

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Dilution Water	9/10	9/10	10/10	10/10	7/10
9510		6/10	8/10	6/10	8/11	8/10
24250		9/10	8/10	8/10	3/10	8/10
59925		8/10	5/10	10/10	8/10	6/10
146500		1/10	1/10	2/10	2/10	3/10
322750		0/10	0/10	0/10	0/10	0/10

(1) DAP 2/18/19 CF

Analyst: [signature] QA: DAP 2/18/19

CETIS Analytical Report

Ephydra cinerea (1)

Report Date: 18 Feb-19 14:00 (p 3 of 3)
Test Code: 474-029Dis | 13-0997-5831

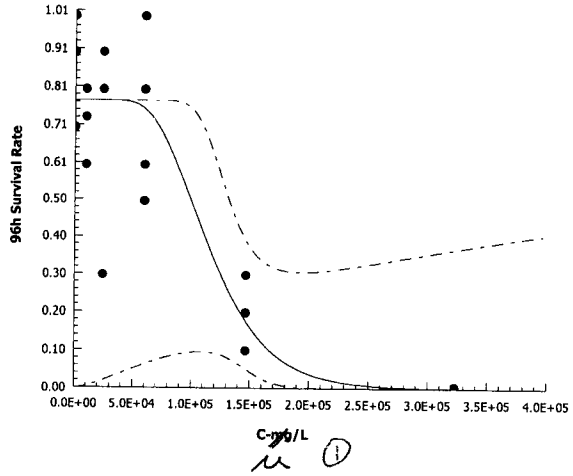
~~Fathead Minnow~~ 96-h Acute Survival Test

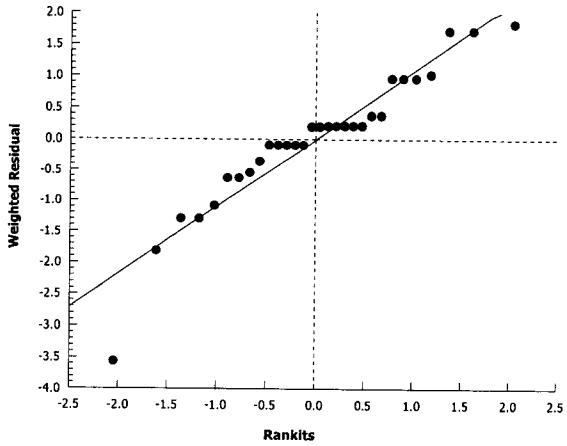
TRE Environmental Strategies, LLC

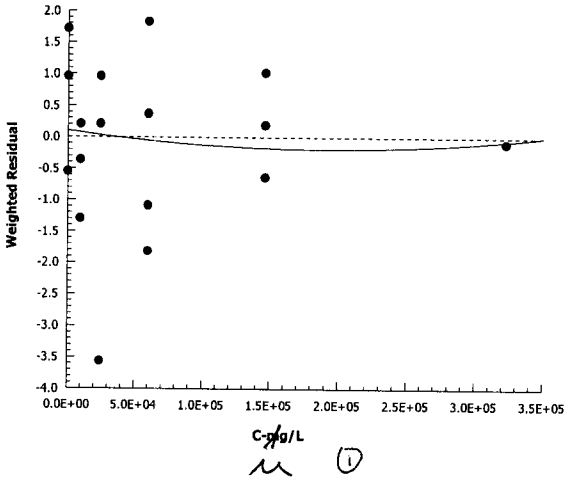
Analysis ID:	09-2725-3283	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Feb-19 14:00	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

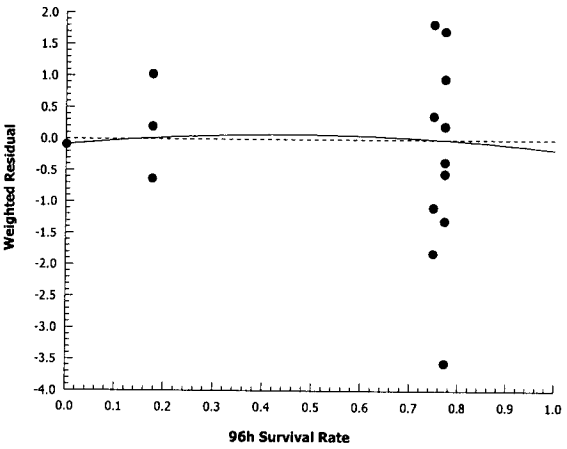
Graphics

Log-Normal [NED=A+B*log(X)]









(1) DAP 2/18/19 CF

Analyst: [signature] QA: DAP 2/18/19